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(54) Title: ANTI-ARTHROPOD VECTOR VACCINES METHODS OF SELECTING AND USES THEREOF

(57) Abstract: The present invention provides methods of selecting and uses of anti-arthropod vector vaccines to prevent Leishmaniases. The present invention also provides compositions for vaccines to prevent Leishmaniases.

ANTI-ARTHROPOD VECTOR VACCINES, METHODS OF SELECTING AND USES THEREOF

BACKGROUND OF THE INVENTION

This application claims priority to U.S. Provisional Application No. 60/299,391, filed June 19, 2001, which application is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to the field of vaccines for the prevention of infectious diseases. Specifically, the present invention relates to the methods of selecting and uses of anti-arthropod vector vaccines to prevent Leishmaniases.

BACKGROUND

The leishmaniases are a group of diseases caused by protozoa of the genus *Leishmania* and affect many millions of people worldwide. In humans, infection with the parasite manifests either as a cutaneous disease caused mainly by *L. major, L. tropica*, and *L. mexicana*; as a mucocutaneous disease caused mainly by *L. brasiliensis*; or as a visceral disease caused mainly by *L. donovani* and *L. chagasi*. All leishmanial diseases are transmitted to their vertebrate hosts by phlebotomine sand flies, which acquire the pathogen by feeding on infected hosts and transmit them by regurgitating the parasite at the site of a subsequent blood meal (1).

While obtaining a blood meal, sand flies salivate into the host's skin. This saliva contains anticlotting, antiplatelet, and vasodilatory compounds that increase the hemorrhagic pool where sand flies feed (2, 3). Some of these components are additionally immunomodulatory. For example, the New World sand fly *Lutzomyia longipalpis* contains the 6.5-kD peptide, maxadilan, which is the most potent vasodilator known (4). Maxadilan additionally has immunosuppressive activities of its own (5), as do many persistent vasodilators such as prostaglandin E_2 (6-8) and calcitonin gene-related peptide (9). Old World sand flies (who share a common

ancestor with New World sand flies before the separation of the present tectonic plates, or about the time of irradiation of mammals) do not have maxadilan but instead use AMP and adenosine as vasodilators (10). Adenosine is also an immunomodulatory component, promoting the production of IL-10 and suppressing TNF-α and IL-12 in mice (11-13). Despite what is known about the role of sandfly saliva and disease transmission, much remains unknown, and an effective vaccine does not exist. Thus, there is a need to prevent infection with the organisms that cause Leishmaniasis. The present invention provides nine major salivary proteins from the sand fly vector of *L. major*, *P. papatasi*, and the nucleic acids that encode them, a vaccine comprising the proteins and/or nucleic acids of the invention, and methods of producing an immune response in a subject to prevent Leishmaniasis.

SUMMARY OF THE INVENTION

The present invention provides an isolated nucleic acid, encoding a salivary polypeptide of *Phlebotomus papatasi*.

The present invention also provides a nucleic acid that hybridizes under stringent conditions to the nucleic acids having SEQ ID NOS:19-27.

The present invention provides a nucleic acid encoding a salivary polypeptide having the amino acid sequence of SEQ ID NOS:10-18.

Also provided by the present invention is an isolated salivary polypeptide of *Phlebotomus papatasi*.

The present invention provides an isolated salivary polypeptide having the N-terminal sequence of SEQ ID NOS:1-9.

The present invention provides a salivary polypeptide having the amino acid sequence of SEQ ID NOS:10-18.

The present invention also provides a vector comprising at least one nucleic acid or fragment thereof, selected from the group consisting of the nucleic acids having SEQ ID NOS:19-27.

The present invention also provides a composition comprising at least one of the vectors and a pharmaceutically acceptable carrier.

The present invention provides a composition comprising at least one salivary polypeptide, or fragment thereof, selected from the group consisting of the polypeptides having SEQ ID NOS:10-18 and a pharmaceutically acceptable carrier.

The present invention also provides a method of producing an immune response in a subject, comprising administering to the subject an effective amount of the various compositions of the invention.

The present invention also provides a method of preventing Leishmaniasis in a subject, comprising administering to the subject an effective amount of the various compositions of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows SDS-PAGE of SGH of *P. papatasi* and Coomassie blue-stained PVDF membrane after gel transfer of 40 homogenized pairs of glands. The numbers represent the position of the mol wt markers.

Figure 1B shows Western blots of the gels with mouse sera obtained from mice immunized by intradermal inoculation of SGH (needle inoculation) or the bite of uninfected sand flies (sand fly bite) with their pre-immunization (PI) controls.

Figure 2 shows SDS-PAGE of 20 homogenized pairs of salivary glands from *P. papatasi*. Left side of figure shows the amino terminal sequence found for each band by Edman degradation; the right side of figure shows the clone identification name. Numbers indicate the position of the mol wt marker in the same gel.

Figure 3A shows the effect on the lesion size of mouse immunization with fractions of SGH separated by SDS-PAGE.

Figure 3B shows the effect of mouse immunization and number of parasites 4.5 wk post challenge following *L. major* infection. Naïve mice were inoculated intradermally with 500 *L. major* promastigotes alone (○) or in the presence of 0.5 pairs of SGH (●). Mice previously vaccinated on the right ear (2-wk intervals) with acrylamide alone, E (■) or mol wt range from 200 to 40 kD, A (◆); 39 to 20 kD, B (◇); or 19 to 3 kD, C (▲) were challenged in the left ear 2 wk following the last immunization with 500 *L. major* promastigotes in the presence of 0.5 pairs of SGH.

The above symbols and bars represent the mean induration in mm ± SE (5 mice per group) or the mean number of parasites per ear ± SE (4 mice per group). (*) indicate significance at $P < 0.05$ when treatment curve (C) was compared with the acrylamide control (E).

Figure 4 shows Western blots of SGH of *P. papatasi* reacted with sera from mice immunized with polyacrylamide fractions separating the SGH by SDS-PAGE. Divisions were a high (A), medium (B), and low (C) mol wt region, as detailed in Fig. 3, or from control mice immunized with polyacrylamide (E), as shown.

Figure 5A shows the effect on the lesion size of mouse immunization with band SP15 from SDS-PAGE gels separating the SGH of *P. papatasi.*

Figure 5B shows the effect of mouse immunization with band SP15 from SDS-PAGE gels separating the SGH of *P. papatasi* on the number of parasites 9 wk post challenge following *L. major* infection. Naïve mice were inoculated intradermally with 500 *L. major* promastigotes alone (○) or in the presence of 0.5 pairs of SGH (●). Mice previously vaccinated on the right ear (2-wk intervals) with acrylamide alone, E (□) or band SP15 (■) were challenged in the left ear 2 wk following the last immunization with 500 *L. major* promastigotes in the presence of 0.5 pairs of SGH. The above symbols and bars represent mean induration in mm ± SE (5 mice per group) or mean number of parasites per ear ± SE (5 mice per group). (*) indicate significance at $P < 0.05$ when the treatment curve was compared with the acrylamide control.

Figure 6A shows that mouse humoral immunity is generated by injection of the VR1020 plasmid containing the SP15 sequence (SP15-Pl). Western blots of salivary homogenates of *P. papatasi* reacted against sera of mice immunized twice in the right ear (2-wk interval) with 5 mg of the SP15-Pl or control VR1020 plasmid (CTL-Pl).

Figure 6B shows that mouse cellular immunity is generated by injection of the VR1020 plasmid containing the SP15 sequence (SP15-Pl). The DTH reaction was induced by sand fly bites on mice vaccinated with SP15-Pl. The left ears of naïve mice, mice immunized with SP15-Pl, or control CTL-Pl mice were exposed to the bite of 10 sand flies. Twenty-four hours later, the mice were sacrificed, and the inflammatory cells (neutrophils, eosinophils, macrophages, dendritic cells, $CD4^+$, and $CD8^+$ T cells)

present in the dermis were analyzed. The symbols and bars represent the mean number of cellular subsets per ear ± SE; 7 mice per group.

Figure 7 shows the effect of mouse immunization with SP15-Pl on the lesion size caused by *L. major* infection. Naïve mice were inoculated intradermally with 500 *L. major* promastigotes alone (○) or in the presence of 0.5 pairs of SGH (●). Mice previously vaccinated on the right ear (2-wk intervals) with SP15-Pl (▫,▪) or CTL-Pl (Δ,▲) were challenged in the left ear 2 wk following the last immunization with 500 *L. major* promastigotes in the presence (▪,▲) or not (▫,Δ) of 0.5 pairs of SGH. The symbols and bars represent the mean induration in mm ± 1 SE; 5 mice per group. (*) indicate significance at $P < 0.05$ when the treatment curve was compared with the control (CTL-Pl + SGH).

Figure 8A shows the effect on lesion size and long-term effect of mouse immunization with SP15-Pl on *L. major* infection. Naïve mice were inoculated intradermally with 500 *L. major* promastigotes alone (○) or in the presence of 0.5 pairs of SGH (●). Mice previously vaccinated on the right ear (2-wk intervals) with SP15-Pl (▫,▪) or CTL-Pl (Δ,▲) were challenged in the left ear 12 wk following the last immunization with 500 *L. major* promastigotes in the presence (▪,▲) or not (▫, Δ) of 0.5 pairs of SGH.

Figure 8B shows the effect on parasite number and long-term effect of mouse immunization with SP15-Pl on *L. major* infection at 6.5 weeks post challenge. The symbols and bars represent the mean induration in mm ± 1 SE; 5 mice per group. (*) indicate significance at $P < 0.05$ when the treatment curve was compared with the control (CTL-Pl + SGH).

Figure 9A shows the humoral response and DTH reaction on $B^{-/-}$ and WT mice following vaccination with SP15-Pl. Western blots showing antibody reactivity of WT but not $B^{-/-}$ mice against *P. papatasi* salivary homogenates are shown.

Figure 9B shows measurements indicating the millimetric difference between the ear challenged with SGH and the non-inoculated ear, on $B^{-/-}$ and WT C57BL/10 mice vaccinated with SP15-Pl. Mice were immunized twice in the right ear (2-wk intervals) or not with 5 mg of SP15-Pl or CTL-Pl and challenged in the left ear 2 wk after the last immunization with 500 *L. major* promastigotes in the presence of 0.5 pairs

of SGH. Twenty-four h after inoculation, the ear thickness was measured and the difference between the ear thickness prior to challenge and 24 h after challenge was computed. Symbols and bars represent mean thickness in mm ± SE; 5 mice per group.

Figure 10A shows lesion size progression and the role of DTH in mouse immunization with SP15-Pl on subsequent *L. major* infection. $B^{-/-}$ mice (Δ,▲) and their controls (C57BL/10, WT) (▫,▪), were immunized twice in the right ear (2-wk interval) with the VR1020 plasmid with (closed symbols) or without (open symbols), the SP15 sequence and challenged 2 wk later in the left ear with *L. major* promastigotes in combination with 0.5 pairs of homogenized *P. papatasi* salivary glands.

Figure 10B shows the role of DTH in mouse immunization with SP15-Pl on subsequent *L. major* infection and parasite numbers recovered from the lesion at 5.5 wk. Each number and bar represents the average ± SE of five mice. (*) indicates significance at $P < 0.05$ when the number of parasites on the SP15-Pl group was compared with the controls of the same mouse group.

DETAILED DESCRIPTION OF THE INVENTION

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a nucleic acid" includes multiple copies of the nucleic acid and can also include more than one particular species of molecule.

The present invention provides an isolated salivary polypeptide of *Phlebotomus papatasi* (*P. papatasi*) and fragments thereof. An "isolated" polypeptide or fragment thereof of this invention is sufficiently free of contaminants or cell components with which polypeptides or fragments thereof normally occur and is present in such concentration as to be the only significant polypeptide or fragment thereof present in the sample. "Isolated" does not mean that the preparation is technically pure (homogeneous), but it is sufficiently pure to provide the polypeptide or fragment thereof in a form in which it can be used therapeutically.

A salivary polypeptide of *P. papatasi* is a polypeptide produced by the salivary glands of the sandfly and secreted into its saliva. As used herein, a "polypeptide" is a chain of amino acids which correspond to those encoded by a nucleic acid. A polypeptide usually describes a chain of amino acids having more than about 30 amino acids. A "fragment" is a specific part of a polypeptide having about 2 to about 30 amino acids. As used herein to describe an amino acid sequence (protein, polypeptide, peptide, etc.), "specific" means that the amino acid sequence is not found identically in any other source. The determination of specificity is made routine, because of the availability of computerized amino acid sequence databases, wherein an amino acid sequence of almost any length can be quickly and reliably checked for the existence of identical sequences. If an identical sequence is not found, the protein is "specific" for the recited source. The term "polypeptide" can refer to a linear chain of amino acids, or it can refer to a chain of amino acids which have been processed and folded into a functional protein. It is understood, however, that 30 is an arbitrary number with regard to distinguishing polypeptides and fragments. The polypeptides and fragments of the present invention are obtained by isolation and purification of the polypeptides and fragments from cells where they are produced naturally or by expression of exogenous nucleic acid encoding the polypeptide or fragment. The polypeptides and fragments of this invention can be obtained by chemical synthesis, by proteolytic cleavage of a polypeptide and/or by synthesis from nucleic acid encoding the polypeptides and fragments.

Examples of an isolated salivary polypeptide of *P. papatasi* include the polypeptides having the N-terminal sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9.

An example of the salivary polypeptide of the invention is the PpSP12 polypeptide. An example of the PpSP12 polypeptide is the PpSP12 polypeptide of *P. papatasi* having an approximate molecular weight of 12 kDa measured in SDS-PAGE under non-reducing conditions. The PpSP12 polypeptide also has an isoelectric point of 9.4.

A further example of the PpSP12 polypeptide is the polypeptide having the N-terminal sequence defined in SEQ ID NO:1. Additional examples of the PpSP12 polypeptide include the polypeptides having at least one of the amino acid sequences of SEQ ID NOS:28-92. A further example of the PpSP12 polypeptide is the polypeptide having the amino acid sequence of SEQ ID NO:10.

An example of the salivary polypeptide of the invention is the PpSP14 polypeptide. An example of the PpSP14 polypeptide is the PpSP14 polypeptide of *P. papatasi* having an approximate molecular weight of 14 kDa measured in SDS-PAGE under non-reducing conditions. The PpSP14 polypeptide also has an isoelectric point of 8.61.

A further example of the PpSP14 polypeptide is the polypeptide having the N-terminal sequence defined in SEQ ID NO:2. Additional examples of the PpSP14 polypeptide include the polypeptides having at least one of the amino acid sequences of SEQ ID NOS:93-142. A further example of the PpSP14 polypeptide is the polypeptide having the amino acid sequence of SEQ ID NO:11.

An example of the salivary polypeptide of the invention is the PpSP15 polypeptide. An example of the PpSP15 polypeptide is the PpSP15 polypeptide of *P. papatasi* having an approximate molecular weight of 15 kDa measured in SDS-PAGE under non-reducing conditions. The PpSP15 polypeptide also has an isoelectric point of 9.43.

A further example of the PpSP15 polypeptide is the polypeptide having the N-terminal sequence defined in SEQ ID NO:3. Additional examples of the PpSP15 polypeptide include the polypeptides having at least one of the amino acid sequences of SEQ ID NOS:143-207. A further example of the PpSP15 polypeptide is the polypeptide having the amino acid sequence of SEQ ID NO:12.

An example of the salivary polypeptide of the invention is the PpSP28 polypeptide. An example of the PpSP28 polypeptide is the PpSP28 polypeptide of *P. papatasi* having an approximate molecular weight of 28 kDa measured in SDS-PAGE under non-reducing conditions. The PpSP28 polypeptide also has an isoelectric point of 8.28.

A further example of the PpSP28 polypeptide is the polypeptide having the N-terminal sequence defined in SEQ ID NO:4. Additional examples of the PpSP28 polypeptide include the polypeptides having at least one of the amino acid sequences of SEQ ID NOS:208-310. A further example of the PpSP28 polypeptide is the polypeptide having the amino acid sequence of SEQ ID NO:13.

An example of the salivary polypeptide of the invention is the PpSP30 polypeptide. An example of the PpSP30 polypeptide is the PpSP30 polypeptide of *P. papatasi* having an approximate molecular weight of 30 kDa measured in SDS-PAGE under non-reducing conditions. The PpSP30 polypeptide also has an isoelectric point of 9.11.

A further example of the PpSP30 polypeptide is the polypeptide having the N-terminal sequence defined in SEQ ID NO:5. Additional examples of the PpSP30 polypeptide include the polypeptides having at least one of the amino acid sequences of SEQ ID NOS:311-424. A further example of the PpSP30 polypeptide is the polypeptide having the amino acid sequence of SEQ ID NO:14.

An example of the salivary polypeptide of the invention is the PpSP32 polypeptide. An example of the PpSP32 polypeptide is the PpSP32 polypeptide of *P. papatasi* having an approximate molecular weight of 32 kDa measured in SDS-PAGE under non-reducing conditions. The PpSP32 polypeptide also has an isoelectric point of 9.26.

A further example of the PpSP32 polypeptide is the polypeptide having the N-terminal sequence defined in SEQ ID NO:6. Additional examples of the PpSP32 polypeptide include the polypeptides having at least one of the amino acid sequences of SEQ ID NOS:425-501. A further example of the PpSP32 polypeptide is the polypeptide having the amino acid sequence of SEQ ID NO:15.

An example of the salivary polypeptide of the invention is the PpSP36 polypeptide. An example of the PpSP36 polypeptide is the PpSP36 polypeptide of *P. papatasi* having an approximate molecular weight of 36 kDa measured in SDS-PAGE under non-reducing conditions. The PpSP36 polypeptide also has an isoelectric point of 8.9.

A further example of the PpSP36 polypeptide is the polypeptide having the N-terminal sequence defined in SEQ ID NO:7. Additional examples of the PpSP36 polypeptide include the polypeptides having at least one of the amino acid sequences of SEQ ID NOS:502-633. A further example of the PpSP36 polypeptide is the polypeptide having the amino acid sequence of SEQ ID NO:16.

An example of the salivary polypeptide of the invention is the PpSP42 polypeptide. An example of the PpSP42 polypeptide is the PpSP42 polypeptide of *P. papatasi* having an approximate molecular weight of 42 kDa measured in SDS-PAGE under non-reducing conditions. The PpSP42 polypeptide also has an isoelectric point of 9.01.

A further example of the PpSP42 polypeptide is the polypeptide having the N-terminal sequence defined in SEQ ID NO:8. Additional examples of the PpSP42 polypeptide include the polypeptides having at least one of the amino acid sequences of SEQ ID NOS:634-759. A further example of the PpSP42 polypeptide is the polypeptide having the amino acid sequence of SEQ ID NO:17.

An example of the salivary polypeptide of the invention is the PpSP44 polypeptide. An example of the PpSP44 polypeptide is the PpSP44 polypeptide of *P. papatasi* having an approximate molecular weight of 44 kDa measured in SDS-PAGE under non-reducing conditions. The PpSP44 polypeptide also has an isoelectric point of 8.70.

A further example of the PpSP44 polypeptide is the polypeptide having the N-terminal sequence defined in SEQ ID NO:9. Additional examples of the PpSP44 polypeptide include the polypeptides having at least one of the amino acid sequences of SEQ ID NOS:760-880. A further example of the PpSP44 polypeptide is the polypeptide having the amino acid sequence of SEQ ID NO:18.

The present invention provides an antigenic or immunogenic fragment of the polypeptides of the invention. "Antigenic" when used herein means capable of binding specifically to an antibody. "Immunogenic" means capable of producing an immune response. The immune response can be humoral and/or cellular; specifically, the immune response can be characterized by the raising of antibodies directed to the antigen and/or characterized by delayed type hypersensitivity. Thus, the polypeptides

and fragments thereof are immunoreactive. As used herein, "immunogenicity" means the ability of a molecule to generate an immune response in a host that reduces the severity of illness when the host is subsequently challenged with the same molecule.

An immunoreactive fragment has an amino acid sequence of at least about 5 consecutive amino acids of a salivary polypeptide amino acid sequence and binds an antibody. An antigenic fragment can be selected by applying the routine technique of epitope mapping to the polypeptides of the present invention to determine the regions of the proteins that contain epitopes reactive with serum antibodies or are immunogenic and capable of eliciting an immune response in an animal. Once the epitope is selected, an antigenic polypeptide containing the epitope can be synthesized directly, or produced recombinantly by cloning nucleic acids encoding the polypeptide in an expression system, according to the standard methods. Alternatively, an antigenic fragment of the antigen can be isolated from the whole antigen or a larger fragment by chemical or mechanical disruption. Fragments can also be randomly chosen from a known salivary polypeptide sequence and synthesized. Two or more fragments that are contiguous in a salivary polypeptide can be combined to form another fragment. The purified fragments thus obtained can be tested to determine their antigenicity and specificity by routine methods. A method of determining immunogenicity is provided in the Examples below.

Polypeptide fragments of the invention include fragments of PpSP12. The fragments can be antigenic or immunogenic fragments of the PpSP12 polypeptide defined by SEQ ID NO:10. Further examples of antigenic or immunogenic (immunoreactive) fragments of the PpSP12 polypeptide include the polypeptide-specific fragments identified in the sequence listing as SEQ ID NOS:28-92.

Polypeptide fragments of the invention include fragments of PpSP14. The fragments can be antigenic or immunogenic fragments of the PpSP14 polypeptide defined by SEQ ID NO:11. Further examples of antigenic or immunogenic (immunoreactive) fragments of the PpSP14 polypeptide include the polypeptide-specific fragments identified in the sequence listing as SEQ ID NOS:93-142.

Polypeptide fragments of the invention include fragments of PpSP15. The fragments can be antigenic or immunogenic fragments of the PpSP15 polypeptide

defined by SEQ ID NO:12. Further examples of antigenic or immunogenic (immunoreactive) fragments of the PpSP15 polypeptide include the polypeptide-specific fragments identified in the sequence listing as SEQ ID NOS:143-207.

Polypeptide fragments of the invention include fragments of PpSP28. The fragments can be antigenic or immunogenic fragments of the PpSP28 polypeptide defined by SEQ ID NO:13. Further examples of antigenic or immunogenic (immunoreactive) fragments of the PpSP28 polypeptide include the polypeptide-specific fragments identified in the sequence listing as SEQ ID NOS:208-310.

Polypeptide fragments of the invention include fragments of PpSP30. The fragments can be antigenic or immunogenic fragments of the PpSP30 polypeptide defined by SEQ ID NO:14. Further examples of antigenic or immunogenic (immunoreactive) fragments of the PpSP30 polypeptide include the polypeptide-specific fragments identified in the sequence listing as SEQ ID NOS:311-424.

Polypeptide fragments of the invention include fragments of PpSP32. The fragments can be antigenic or immunogenic fragments of the PpSP32 polypeptide defined by SEQ ID NO:15. Further examples of antigenic or immunogenic (immunoreactive) fragments of the PpSP32 polypeptide include the polypeptide-specific fragments identified in the sequence listing as SEQ ID NOS:425-501.

Polypeptide fragments of the invention include fragments of PpSP36. The fragments can be antigenic or immunogenic fragments of the PpSP36 polypeptide defined by SEQ ID NO:16. Further examples of antigenic or immunogenic (immunoreactive) fragments of the PpSP36 polypeptide include the polypeptide-specific fragments identified in the sequence listing as SEQ ID NOS:502-633.

Polypeptide fragments of the invention include fragments of PpSP42. The fragments can be antigenic or immunogenic fragments of the PpSP42 polypeptide defined by SEQ ID NO:17. Further examples of antigenic or immunogenic (immunoreactive) fragments of the PpSP42 polypeptide include the polypeptide-specific fragments identified in the sequence listing as SEQ ID NOS:634-759.

Polypeptide fragments of the invention include fragments of PpSP44. The fragments can be antigenic or immunogenic fragments of the PpSP44 polypeptide defined by SEQ ID NO:18. Further examples of antigenic or immunogenic

(immunoreactive) fragments of the PpSP44 polypeptide include the polypeptide-specific fragments identified in the sequence listing as SEQ ID NOS:760-880.

Modifications to any of the above polypeptides or fragments can be made, while preserving the specificity and activity (function) of the native polypeptide or fragment thereof. As used herein, "native" describes a protein that occurs in nature. The modifications contemplated herein can be conservative amino acid substitutions, for example, the substitution of a basic amino acid for a different basic amino acid. Modifications can also include creation of fusion proteins with epitope tags or known recombinant proteins or genes encoding them created by subcloning into commercial or non-commercial vectors (e.g., polyhistidine tags, flag tags, myc tag, glutathione-S-transferase [GST] fusion protein, *xylE* fusion reporter construct). Furthermore, the modifications contemplated will not affect the function of the polypeptide or the way the polypeptide accomplishes that function (e.g., its secondary structure or the ultimate result of the polypeptide's activity. These products are equivalent to the salivary polypeptides of the present invention. The means for determining these parameters are well known.

The present invention provides an isolated nucleic acid, encoding a salivary polypeptide of *Phlebotomus papatasi* (*P. papatasi*). "Nucleic acid" as used herein refers to single- or double-stranded molecules which may be DNA, comprised of the nucleotide bases A, T, C and G, or RNA, comprised of the bases A, U (substitutes for T) , C, and G. The nucleic acid may represent a coding strand or its complement. Nucleic acids may be identical in sequence to the sequence which is naturally occurring or may include alternative codons which encode the same amino acid as that which is found in the naturally occurring sequence. Furthermore, nucleic acids may include codons which represent conservative substitutions of amino acids as are well known in the art. Such nucleic acids can be used as probes and primers of the present invention.

As used herein, the term "isolated nucleic acid" means a nucleic acid separated or substantially free from at least some of the other components of the naturally occurring organism, for example, the cell structural components commonly found associated with nucleic acids in a cellular environment and/or other nucleic acids of the organism. The isolation of nucleic acids can therefore be accomplished by techniques

such as cell lysis followed by phenol plus chloroform extraction, followed by ethanol precipitation of the nucleic acids. The nucleic acids of this invention can be isolated from cells according to methods well known in the art for isolating nucleic acids. Alternatively, the nucleic acids of the present invention can be synthesized according to standard protocols well described in the literature for synthesizing nucleic acids. Modifications to the nucleic acids of the invention are also contemplated, provided that the essential structure and function of the peptide or polypeptide encoded by the nucleic acid are maintained.

The nucleic acid of this invention can be part of a recombinant nucleic acid construct comprising any combination of restriction sites and/or functional elements as are well known in the art which facilitate molecular cloning and other recombinant DNA manipulations. Thus, the present invention further provides a recombinant nucleic acid construct comprising a nucleic acid encoding a polypeptide or fragment thereof of this invention.

Salivary polypeptide-encoding nucleic acids can be isolated from an organism in which they are normally found (e.g., *P. papatasi*), using any of the routine techniques. For example, a genomic DNA or cDNA library can be constructed and screened for the presence of the nucleic acid of interest using one of the present salivary gland protein nucleic acids as a probe. Methods of constructing and screening such libraries are well known in the art and kits for performing the construction and screening steps are commercially available (for example, Stratagene Cloning Systems, La Jolla, CA). Once isolated, the nucleic acid can be directly cloned into an appropriate vector, or if necessary, can be modified to facilitate the subsequent cloning steps. Such modification steps are routine, an example of which is the addition of oligonucleotide linkers which contain restriction sites to the termini of the nucleic acid. General methods are set forth in Sambrook et al.

Salivary polypeptide-encoding nucleic acids can also be synthesized. For example, a method of obtaining a DNA molecule encoding a specific salivary polypeptide is to synthesize a recombinant DNA molecule which encodes the polypeptide. For example, oligonucleotide synthesis procedures are routine in the art and oligonucleotides coding for a particular protein region are readily obtainable

through automated DNA synthesis. A nucleic acid for one strand of a double-stranded molecule can be synthesized and hybridized to its complementary strand. One can design these oligonucleotides such that the resulting double-stranded molecule has either internal restriction sites or appropriate 5' or 3' overhangs at the termini for cloning into an appropriate vector. Double-stranded molecules coding for relatively large proteins can readily be synthesized by first constructing several different double-stranded molecules that code for particular regions of the protein, followed by ligating these DNA molecules together. In the Examples below, another method for isolating the nucleic acids and fragments thereof is taught.

Examples of nucleic acids of the invention include those that encode a salivary polypeptide having the N-terminal sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9.

The present invention also provides a nucleic acid encoding a salivary polypeptide of *P. papatasi*, wherein the salivary polypeptide has the amino acid sequence selected from the group consisting of SEQ ID NO:10 (PpSP12), SEQ ID NO:11 (PpSP14), SEQ ID NO:12 (PpSP15), SEQ ID NO:13 (PpSP28), SEQ ID NO:14 (PpSP30), SEQ ID NO:15 (PpSP32), SEQ ID NO:16 (PpSP36), SEQ ID NO:17 (PpSP42) and SEQ ID NO:18 (PpSP44).

A nucleic acid that encodes a salivary polypeptide of the invention includes the nucleic acid that encodes PpSP12. A specific example of a nucleic acid that encodes PpSP12 is the nucleic acid of SEQ ID NO:19. The nucleotide sequence of PpSP12 is nucleotide 21 through 443.

A nucleic acid that encodes a salivary polypeptide of the invention includes the nucleic acid that encodes PpSP14. A specific example of a nucleic acid that encodes PpSP14 is the nucleic acid of SEQ ID NO:20. The nucleotide sequence of PpSP14 is nucleotide 15 through 444.

A nucleic acid that encodes a salivary polypeptide of the invention includes the nucleic acid that encodes PpSP15. A specific example of a nucleic acid that encodes PpSP15 is the nucleic acid of SEQ ID NO:21. The nucleotide sequence of PpSP15 is nucleotide 18 through 446.

A nucleic acid that encodes a salivary polypeptide of the invention includes the nucleic acid that encodes PpSP28. A specific example of a nucleic acid that encodes PpSP28 is the nucleic acid of SEQ ID NO:22. The nucleotide sequence of PpSP28 is nucleotide 19 through 783.

A nucleic acid that encodes a salivary polypeptide of the invention includes the nucleic acid that encodes PpSP30. A specific example of a nucleic acid that encodes PpSP30 is the nucleic acid of SEQ ID NO:23. The nucleotide sequence of PpSP30 is nucleotide 29 through 790.

A nucleic acid that encodes a salivary polypeptide of the invention includes the nucleic acid that encodes PpSP32. A specific example of a nucleic acid that encodes PpSP32 is the nucleic acid of SEQ ID NO:24. The nucleotide sequence of PpSP32 is nucleotide 29 through 769.

A nucleic acid that encodes a salivary polypeptide of the invention includes the nucleic acid that encodes PpSP36. A specific example of a nucleic acid that encodes PpSP36 is the nucleic acid of SEQ ID NO:25. The nucleotide sequence of PpSP36 is nucleotide 43 through 1053.

A nucleic acid that encodes a salivary polypeptide of the invention includes the nucleic acid that encodes PpSP42. A specific example of a nucleic acid that encodes PpSP42 is the nucleic acid of SEQ ID NO:26. The nucleotide sequence of PpSP42 is nucleotide 25 through 1212.

A nucleic acid that encodes a salivary polypeptide of the invention includes the nucleic acid that encodes PpSP44. A specific example of a nucleic acid that encodesPpSP44 is the nucleic acid of SEQ ID NO:27. The nucleotide sequence of PpSP44 is nucleotide 23 through 1225.

Having provided and taught how to obtain a nucleic acid that encodes a salivary polypeptide, an isolated nucleic acid that encodes a fragment of a polypeptide is also provided. The fragment can be obtained using any of the methods applicable to the full gene. The fragment can encode a protein specific fragment (i.e., found in a salivary polypeptide, but not in other proteins) and/or a species-specific fragment (e.g., found in the salivary polypeptides of *P. papatasi*, but not in the salivary polypeptides of other species). Nucleic acids encoding protein-specific and/or species-specific fragments of

salivary polypeptides are themselves gene-specific, species-specific or allele-specific fragments of the genes encoding the polypeptides and fragments of the present invention.

As used herein, a "fragment of a nucleic acid" is a specific part of a nucleic acid having at least about 6 nucleotides. As used herein to describe a nucleic acid sequence, "specific" means that the nucleic acid sequence is not found identically in any other source. The determination of specificity is made routine because of the availability of computerized nucleic acid sequence databases, wherein a nucleic acid sequence of almost any length can be quickly and reliably checked for the existence of identical sequences. If an identical sequence is not found, the nucleic acid fragment is "specific" for the recited source.

A nucleic acid fragment of the invention can be a nucleic acid that encodes a fragment of PpSP12. The fragment can encode a polypeptide fragment specific for the polypeptide having the amino acid sequence of SEQ ID NO:10. A specific example of a fragment that encodes PpSP12 is the nucleic acid comprising nucleotides 81 through 137 of SEQ ID NO:19 that encodes the amino acid sequence of SEQ ID NO:1. Further examples of nucleic acid fragments of the invention include the nucleic acids that encode fragments of the PpSP12 polypeptide defined as SEQ ID NOS:28-92.

A nucleic acid fragment of the invention can be a nucleic acid that encodes a fragment of PpSP14. The fragment can encode a polypeptide fragment specific for the polypeptide having the amino acid sequence of SEQ ID NO:11. A specific example of a fragment that encodes PpSP14 is the nucleic acid comprising nucleotides 75 through 128 of SEQ ID NO:20 that encodes the amino acid sequence of SEQ ID NO:2. Further examples of nucleic acid fragments of the invention include the nucleic acids that encode fragments of the PpSP14 polypeptide defined as SEQ ID NOS:93-142.

A nucleic acid fragment of the invention can be a nucleic acid that encodes a fragment of PpSP15. The fragment can encode a polypeptide fragment specific for the polypeptide having the amino acid sequence of SEQ ID NO:12. A specific example of a fragment that encodes PpSP15 is the nucleic acid comprising nucleotides 78 through 134 of SEQ ID NO:21 that encodes the amino acid sequence of SEQ ID NO:3. Further

examples of nucleic acid fragments of the invention include the nucleic acids that encode fragments of the PpSP15 polypeptide defined as SEQ ID NOS:143-207.

A nucleic acid fragment of the invention can be a nucleic acid that encodes a fragment of PpSP28. The fragment can encode a polypeptide fragment specific for the polypeptide having the amino acid sequence of SEQ ID NO:13. A specific example of a fragment that encodes PpSP28 is the nucleic acid comprising nucleotides 76 through 132 of SEQ ID NO:22 that encodes the amino acid sequence of SEQ ID NO:4. Further examples of nucleic acid fragments of the invention include the nucleic acids that encode fragments of the PpSP28 polypeptide defined as SEQ ID NOS:208-310.

A nucleic acid fragment of the invention can be a nucleic acid that encodes a fragment of PpSP30. The fragment can encode a polypeptide fragment specific for the polypeptide having the amino acid sequence of SEQ ID NO:14. A specific example of a fragment that encodes PpSP30 is the nucleic acid comprising nucleotides 86 through 133 of SEQ ID NO:23 that encodes the amino acid sequence of SEQ ID NO:5. Further examples of nucleic acid fragments of the invention include the nucleic acids that encode fragments of the PpSP30 polypeptide defined as SEQ ID NOS:311-424.

A nucleic acid fragment of the invention can be a nucleic acid that encodes a fragment of PpSP32. The fragment can encode a polypeptide fragment specific for the polypeptide having the amino acid sequence of SEQ ID NO:15. A specific example of a fragment that encodes PpSP32 is the nucleic acid comprising nucleotides 98 through 139 of SEQ ID NO:24 that encodes the amino acid sequence of SEQ ID NO:6. Further examples of nucleic acid fragments of the invention include the nucleic acids that encode fragments of the PpSP32 polypeptide defined as SEQ ID NOS:425-501.

A nucleic acid fragment of the invention can be a nucleic acid that encodes a fragment of PpSP36. The fragment can encode a polypeptide fragment specific for the polypeptide having the amino acid sequence of SEQ ID NO:16. A specific example of a fragment that encodes PpSP36 is the nucleic acid comprising nucleotides 106 through 162 of SEQ ID NO:25 that encodes the amino acid sequence of SEQ ID NO:7. Further examples of nucleic acid fragments of the invention include the nucleic acids that encode fragments of the PpSP36 polypeptide defined as SEQ ID NOS:502-633.

A nucleic acid fragment of the invention can be a nucleic acid that encodes a fragment of PpSP42. The fragment can encode a polypeptide fragment specific for the polypeptide having the amino acid sequence of SEQ ID NO:17. A specific example of a fragment that encodes PpSP42 is the nucleic acid comprising nucleotides 82 through 135 of SEQ ID NO:26 that encodes the amino acid sequence of SEQ ID NO:8. Further examples of nucleic acid fragments of the invention include the nucleic acids that encode fragments of the PpSP42 polypeptide defined as SEQ ID NOS:634-759.

A nucleic acid fragment of the invention can be a nucleic acid that encodes a fragment of PpSP44. The fragment can encode a polypeptide fragment specific for the polypeptide having the amino acid sequence of SEQ ID NO:18. A specific example of a fragment that encodes PpSP44 is the nucleic acid comprising nucleotides 77 through 136 of SEQ ID NO:27 that encodes the amino acid sequence of SEQ ID NO:9. Further examples of nucleic acid fragments of the invention include the nucleic acids that encode fragments of the PpSP44 polypeptide defined as SEQ ID NOS:760-880.

The present invention provides a nucleic acid of at least 10 nucleotides that hybridizes under stringent conditions to the nucleic acids that encode the salivary polypeptides and fragments of the present invention. For example, the conditions can be polymerase chain reaction conditions and the hybridizing nucleic acid can be a primer consisting of a specific fragment of the reference sequence or a nearly identical nucleic acid that hybridizes only to the exemplified salivary polypeptide gene or a homolog thereof.

The invention provides an isolated nucleic acid that specifically hybridizes with the salivary polypeptide-encoding genes shown in the sequence set forth as SEQ ID NOS:19-27 under the conditions of about 16 hrs at about 65°C, about 5x SSC, about 0.1% SDS, about 2x Denhardt's solution, about 150 μg/ml salmon sperm DNA with washing at about 65°C, 30 min, 2x, in about 0.1x SSPE/o.1% SDS. Alternative hybridization conditions include 68°C for about 16 hours in buffer containing about 6X SSC, 0.5% sodium dodecyl sulfate, about 5X Denhardt's solution and about 100μg salmon sperm DNA, with washing at about 60°C in about 0.5X SSC. For example, the hybridizing nucleic acid can be a probe that hybridizes only to the exemplified salivary polypeptide gene or a homolog thereof. Thus, the hybridizing nucleic acid can be a

naturally occurring homolog of the exemplified genes. The hybridizing nucleic acid can also include insubstantial base substitutions that do not prevent hybridization under the stated conditions or affect the function of the encoded protein, the way the protein accomplishes that function (e.g., its secondary structure or the ultimate result of the protein's activity. The means for determining these parameters are well known.

As used herein to describe nucleic acids, the term "selectively hybridizes" excludes the occasional randomly hybridizing nucleic acids as well as nucleic acids that encode other known homologs of the present polypeptides. The selectively hybridizing nucleic acids of the invention can have at least 70%, 73%, 78%, 80%, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% and 99% complementarity with the segment and strand of the sequence to which it hybridizes. The nucleic acids can be at least 10, 18, 20, 25, 50, 100, 150, 200, 300, 500, 550, 750, 900, 950, or 1000 nucleotides in length, depending on whether the nucleic acid is to be used as a primer, probe or for protein expression. Thus, the nucleic acid can be an alternative coding sequence for the protein, or can be used as a probe or primer for detecting the presence of a salivary polypeptide-encoding nucleic acid or obtaining such polypeptide. If used as primers, the invention provides compositions including at least two nucleic acids which selectively hybridize with different regions so as to amplify a desired region. Depending on the length of the probe or primer, it can range between 70% complementary bases and full complementarity and still hybridize under stringent conditions. For example, for the purpose of diagnosing the presence of a salivary polypeptide-encoding nucleic acid, the degree of complementarity between the hybridizing nucleic acid (probe or primer) and the sequence to which it hybridizes (DNA from a sample) should be at least enough to exclude hybridization with a nucleic acid from a related organism. The invention provides examples of these nucleic acids so that the degree of complementarity required to distinguish selectively hybridizing from nonselectively hybridizing nucleic acids under stringent conditions can be clearly determined for each nucleic acid. It should also be clear that the hybridizing nucleic acids of the invention will not hybridize with nucleic acids encoding unrelated proteins (hybridization is selective) under stringent conditions.

"Stringent conditions" refers to the washing conditions used in a hybridization protocol. In general, the washing conditions should be a combination of temperature and salt concentration chosen so that the denaturation temperature is approximately 5-20°C below the calculated T_m of the hybrid under study. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to the probe or protein coding nucleic acid of interest and then washed under conditions of different stringencies. For example, $MgCl_2$ concentrations used in PCR buffer can be altered to increase the specificity with which the primer binds to the template, but the concentration range of this compound used in hybridization reactions is narrow, and therefore, the proper stringency level is easily determined. For example, hybridizations with oligonucleotide probes 18 nucleotides in length can be done at 5-10°C below the estimated T_m in 6X SSPE, then washed at the same temperature in 2X SSPE. The T_m of such an oligonucleotide can be estimated by allowing 2°C for each A or T nucleotide, and 4°C for each G or C. An 18 nucleotide probe of 50% G+C would, therefore, have an approximate T_m of 54°C. Likewise, the starting salt concentration of an 18 nucleotide primer or probe would be about 100-200 mM. Thus, stringent conditions for such an 18 nucleotide primer or probe would be a T_m of about 54°C and a starting salt concentration of about 150 mM and modified accordingly by preliminary experiments. T_m values can also be calculated for a variety of conditions utilizing commercially available computer software (e.g., OLIGO®).

Once a nucleic acid encoding a particular polypeptide of interest, or a region of that nucleic acid, is constructed, modified, or isolated, that nucleic acid can then be cloned into an appropriate vector, which can direct the *in vivo* or *in vitro* synthesis of that wild-type and/or modified polypeptide. The vector is contemplated to have the necessary functional elements that direct and regulate transcription of the inserted gene, or hybrid gene. These functional elements include, but are not limited to, a promoter, regions upstream or downstream of the promoter, such as enhancers that may regulate the transcriptional activity of the promoter, an origin of replication, appropriate restriction sites to facilitate cloning of inserts adjacent to the promoter, antibiotic resistance genes or other markers which can serve to select for cells containing the

vector or the vector containing the insert, RNA splice junctions, a transcription termination region, or any other region which may serve to facilitate the expression of the inserted gene or hybrid gene. (See generally, Sambrook *et al.*). Thus, the nucleic acids of the present invention can be in a vector, and the vector can be in a host for expressing the nucleic acid.

The present invention provides a vector comprising at least one nucleic acid or fragment thereof encoding a polypeptide or fragment thereof of this invention. The vector can be an expression vector which contains all of the genetic components required for expression of the nucleic acid in cells into which the vector has been introduced, as are well known in the art. The expression vector can be a commercial expression vector or it can be constructed in the laboratory according to standard molecular biology protocols. The expression vector can comprise viral nucleic acid including, but not limited to, vaccinia virus, adenovirus, retrovirus and/or adeno-associated virus nucleic acid. The nucleic acid or vector of this invention can also be in a liposome or a delivery vehicle which can be taken up by a cell via receptor-mediated or other type of endocytosis.

The nucleic acid of this invention can be in a cell, which can be a cell expressing the nucleic acid whereby a polypeptide or fragment thereof of this invention is produced in the cell. In addition, the vector of this invention can be in a cell, which can be a cell expressing the nucleic acid of the vector whereby a polypeptide or fragment thereof of this invention is produced in the cell. It is also contemplated that the nucleic acids and/or vectors of this invention can be present in a host animal (e.g., a transgenic animal) which expresses the nucleic acids of this invention and produces the polypeptides or fragments thereof of this invention.

The present invention provides a composition comprising at least one vector of the present invention and a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected peptide, polypeptide, nucleic acid, vector or cell without causing substantial deleterious biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained.

The present invention also provides a composition comprising a salivary polypeptide or fragment thereof and a pharmaceutically acceptable carrier. Also provided is a composition comprising at least one polypeptide or fragment, selected from the group consisting of the polypeptides having SEQ ID NOS:10-18 and fragments having the SEQ ID NOS:29-880.

Furthermore, any of the compositions of this invention can comprise in addition to a pharmaceutically acceptable carrier a suitable adjuvant. As used herein, "suitable adjuvant" describes an adjuvant capable of being combined with the polypeptide or fragment thereof of this invention to further enhance an immune response without deleterious effect on the subject or the cell of the subject. A suitable adjuvant can be, but is not limited to, MONTANIDE ISA51 (Seppic, Inc., Fairfield, NJ), SYNTEX adjuvant formulation 1 (SAF-1), composed of 5 percent (wt/vol) squalene (DASF, Parsippany, N.J.), 2.5 percent Pluronic, L121 polymer (Aldrich Chemical, Milwaukee), and 0.2 percent polysorbate (Tween 80, Sigma) in phosphate-buffered saline. Other suitable adjuvants are well known in the art and include QS-21, Freund's adjuvant (complete and incomplete), alum, aluminum phosphate, aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), –acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE) and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trealose dimycolate and cell wall skeleton (MPL+TDM+CWS) in 2% squalene/Tween 80 emulsion.

The present invention provides a method of producing an immune response in a subject, comprising administering to the subject an effective amount of at least one composition of the present invention and a pharmaceutically acceptable carrier. In general, an " effective amount" of an agent is that amount needed to achieve the desired result or results. Detection of an immune response in the subject or in the cells of the subject can be carried out according to the methods set forth in the Examples provided herein, such as detecting the presence of delayed type hypersensitivity activated by the polypeptides or fragments thereof of this invention.

As used throughout, by a "subject" is meant an individual. Thus, the "subject" can include domesticated animals, such as cats, dogs, etc., livestock (e.g., cattle, horses, pigs, sheep, goats, etc.), laboratory animals (e.g., mouse, rabbit, rat, guinea pig, etc.) and birds. Preferably, the subject is a mammal such as a primate, and, more preferably, a human.

The present invention also provides a method of preventing Leishmaniasis in a subject, comprising administering to the subject an effective amount of at least one composition of the present invention and a pharmaceutically acceptable carrier.

In the methods in which the composition comprises a nucleic acid, delivery of the nucleic acid or vector to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector of this invention can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

As one example, vector delivery can be via a viral system, such as a retroviral vector system which can package a recombinant retroviral genome. The recombinant retrovirus can then be used to infect and thereby deliver to the infected cells nucleic acid encoding the peptide or polypeptide. The exact method of introducing the exogenous nucleic acid into mammalian cells is, of course, not limited to the use of retroviral vectors. Other techniques are widely available for this procedure including the use of adenoviral vectors, adeno-associated viral (AAV) vectors, lentiviral vectors, pseudotyped retroviral vectors and vaccinia viral vectors, as well as any other viral vectors now known or developed in the future. Physical transduction techniques can also be used, such as liposome delivery and receptor-mediated and other endocytosis mechanisms. This invention can be used in conjunction with any of these or other commonly used gene transfer methods.

As one example, if the nucleic acid of this invention is delivered to the cells of a subject in an adenovirus vector, the dosage for administration of adenovirus to humans can range from about 10^7 to 10^9 plaque forming units (pfu) per injection, but can be as high as 10^{12} pfu per injection. Ideally, a subject will receive a single injection. If additional injections are necessary, they can be repeated at intervals (1-6 months) for an indefinite period and/or until the efficacy of the treatment has been established. As set forth herein, the efficacy of treatment can be determined by evaluating the clinical parameters described herein. Efficacy of treatment is measured by absence of disease in subjects exposed to the pathogen.

The exact amount of the nucleic acid or vector required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every nucleic acid or vector. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

The dosage of the composition varies depending on the weight, age, sex, and method of administration. The dosage can also be adjusted by the individual physician as called for based on the particular circumstances. The compositions can be administered conventionally as vaccines containing the active composition as a predetermined quantity of active material calculated to produce the desired therapeutic or immunologic effect in association with the required pharmaceutically acceptable carrier or diluent (*i.e.*, carrier or vehicle). For example, 50 μg of a DNA construct vaccine of the present invention can be injected intradermally three times at two week intervals to produce the desired therapeutic or immunologic effect. In another embodiment, a 1 mg/Kg dosage of a protein vaccine of the present invention can be injected intradermally three times at two week intervals to produce the desired therapeutic or immunologic effect.

Parenteral administration of the polypeptides or fragments thereof, nucleic acids and/or vectors of the present invention, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to

injection, or as emulsions. As used herein, "parenteral administration" includes intradermal, subcutaneous, intramuscular, intraperitoneal, intravenous and intratracheal routes. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

The present invention is more particularly described in the following examples which are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art.

EXAMPLES

Sand Flies and Preparation of SGH. *P. papatasi*, Israeli strain, were reared at the Walter Reed Army Medical Research Institute, using as larval food a mixture of fermented rabbit feces and rabbit food (20). Adult sand flies were offered a cotton swab containing 20% sucrose and were used for dissection of salivary glands at 2-7d following emergence. Salivary glands were stored in groups of 20 pairs in 20 ml NaCl (150 mM) Hepes buffer (10 mM, pH 7.4). Salivary glands were disrupted by ultrasonication within 1.5-ml conical tubes. Tubes were centrifuged at 10,000 *g* for 2 min and the resultant supernatant used for the studies.

Mice. C57BL/6 mice were purchased from the Division of Cancer Treatment, National Cancer Institute. $B^{-/-}$ mice were obtained from Taconic Farms. Mice were maintained in the NIAID Animal Care Facility under pathogen-free conditions.

SDS-PAGE. Tris-glycine gels (16%), 1 mm thick, were used (Invitrogen). Gels were run with Tris-glycine buffer according to the manufacturer's instructions. To estimate the mol wt of the samples, SeeBlue™ markers from Invitrogen (myosin, BSA, glutamic dehydrogenase, alcohol dehydrogenase, carbonic anhydrase, myoglobin, lysozyme, aprotinin, and insulin, chain B) were used. SGH were treated with equal parts of 2X SDS sample buffer (8% SDS in Tris-HCl buffer, 0.5M, pH 6.8, 10% glycerol and 1% bromophenol blue dye). Thirty pairs of homogenized salivary glands per lane (approximately 30mg protein) were applied when visualization of the protein bands stained with Coomassie blue was desired. For amino terminal sequencing of the

salivary proteins, 40 homogenized pairs of glands were electrophoresed and transferred to polyvinylidene difluoride (PVDF) membrane using 10 mM CAPS, pH 11, 10% methanol as the transfer buffer on a Blot-Module for the XCell II Mini-Cell (Invitrogen). The membrane was stained with Coomassie blue in the absence of acetic acid. Stained bands were cut from the PVDF membrane and subjected to Edman degradation using a Procise sequencer (Perkin-Elmer Corp.).

The salivary gland of *P. papatasi* is a sac-like structure consisting of a unicellular epithelium layer surrounding a large lumen (28). After a blood meal, the gland total protein content decreases to half or less from its ~1μg value (29). Accordingly, most of the protein from the fly SGH must be destined for secretion. Indeed, SDS-PAGE of SGH reveals a low complexity composition consisting of ~12 major bands varying from 10-100 kD (Fig. 1 A).

Western Blot Analysis. SDS-PAGE of *P. papatasi* SGH (50 salivary gland pairs) for Western blot analysis was done on 16% Tris-glycine gel containing a single long well (Invitrogen). After electrophoresis, salivary proteins were transferred to a nitrocellulose membrane using Tris-glycine buffer with 10% methanol as the transfer buffer on a Blot-Module for the XCell II Mini-Cell. The nitrocellulose membrane was then incubated overnight at 4°C with blocking buffer (Tris pH 8.0 plus 150 mM NaCl plus 5% non-fat milk). Following the blocking step, the membrane was placed on a Mini-protean II multi-screen apparatus (Bio-Rad) that allows testing of 16 different serum samples from a single blot. Sera from mice pre-exposed to SGH, from mice bitten by sand flies or from naive mice, were diluted 1:200 with blocking buffer and added individually to various channels on the multi screen apparatus. The membrane was incubated with the different serum samples for 2 h at room temperature. Serum samples were removed from the channels of the multi screen apparatus and washing buffer (Tris, pH 8.0 plus 150 mM NaCl plus 0.1% Tween 20) was added three times. The membrane was then removed from the multi screen device and washed again three times, 5 min per wash, with washing buffer. The membrane was then incubated with a 1:10,000 dilution of an anti-mouse IgG alkaline phosphatase-conjugated antibody for 1 h at room temperature. The membrane was washed three times with washing buffer as described above. Positive bands were visualized by adding alkaline phosphatase

substrate (Promega) and the reaction stopped by washing the membrane three times with deionized water.

Sera from individual mice inoculated with SGH or exposed to sand fly bites recognized antigens that co-localized with one or more of these major bands when detected by Western blot assays (Fig. 1 B). Non-exposed controls or pre-immune sera did not react to *P. papatasi* salivary antigens (Fig. 1 B).

Salivary Gland cDNA Library Construction. P. papatasi salivary gland mRNA was isolated from eighty-five salivary gland pairs from adult females, at days 1, 2 and 3 after emergence. The Micro-FastTrack mRNA isolation kit (Invitrogen, San Diego, CA) was used, yielding a total of 100 ng poly (A)+ mRNA. The PCR-based cDNA library was made following the instructions for the SMART cDNA library construction kit (Clontech, Palo Alto, CA). One hundred nanograms of *P. papatasi* salivary gland mRNA was reverse transcribed to cDNA using Superscript II RNase H- reverse transcriptase (Gibco-BRL, Gaithersburg, MD) and the CDS/3' primer (Clontech, Palo Alto, CA) for 1 hr at 42°C. Second strand synthesis was performed using a PCR-based protocol by using the SMART III primer (Clontech, Palo Alto, CA) as the sense primer and the CDS/3' primer as anti-sense primer, these two primers additionally, create at the ends of the nascent cDNA SfiI A and B sites respectively. Double strand cDNA synthesis was done on a Perkin Elmer 9700 Thermal cycler (Perkin Elmer Corp., Foster City, CA) and using the Advantage Klen-Taq DNA polymerase (Clontech, Palo Alto, CA). PCR conditions were the following: 94°C for 2 min; 19 cycles of 94°C for 10 sec and 68°C for 6 min. Double strand cDNA was immediately treated with proteinase K (0.8 μg/μl) for 20 minutes at 45°C and washed three times with water using Amicon filters with a 100 kD cut off (Millipore Corp., Bedford MA). The double strand cDNA was then digested with Sfi I for 2 hours at 50°C (The Sfi I sites were inserted to the cDNA during the second strand synthesis using the SMART III and the CDS/3' primer). The cDNA was then fractionated using columns provided by the manufacturer (Clontech, Palo Alto, CA). Fractions containing cDNA of more than 400 bp were pooled, concentrated and washed three times with water using an Amicon filter with a 100 kD cut-off. The cDNA was concentrated to a volume of 7 μl. The concentrated cDNA was then ligated into a lambda triplex2

vector (Clontech, Palo Alto, CA), and the resulting ligation reaction was packed using the Gigapack gold III from Stratagene/Biocrest (Cedar Creek, TE) following manufacturer's specifications. The obtained library was plated by infecting log phase XL1- blue cells (Clontech, Palo Alto, CA) and the amount of recombinants was determined by PCR using vector primers flanking the inserted cDNA and visualized on a 1.1 % agarose gel with ethidium bromide (1.5 μg/ml).

Massive Sequencing of P. papatasi Salivary Gland cDNA Library. P. papatasi salivary gland cDNA library was plated to ca. 200 plaques per plate (150 mm Petri dish). The plaques were randomly picked and transferred to a 96 well polypropylene plate containing 100 μl of water per well. The plate was covered and placed on a gyrator shaker for 1 hr at room temperature. Five microliters of the phage sample was used as a template for a PCR reaction to amplify random cDNAs. The primers used for this reaction were sequences from the triplEX2 vector, the primers were named PT2F1 (5'- AAGTACTCT AGCAAT TGTGAGC- 3') (SEQ ID NO:881) which is positioned upstream of the cDNA of interest (5' end) , and PT2R1 (5'- CTCTTCGCTATTACGCCAGCT G- 3') (SEQ ID NO:882) which is positioned downstream of the cDNA of interest (3' end). High fidelity platinum Taq polymerase (Gibco-BRL, Gaithersburg, MD) was used for these reactions. Amplification conditions were: 1 hold of 75°C for 3 minutes, 1 hold of 94°C for 3 minutes and 34 cycles of 94°C for 30 sec, 49°C for 30sec and 72°C for 1 min and 20 sec. Amplified products were visualized on a 1.1% agarose gel with ethidium bromide. The concentration of double strand cDNA was measured by using the Hoechst dye 33258 on a Fluorite 1000 plate fluorometer (Dynatech Laboratories, Chantilly,VA). Three to four microliters of PCR reaction containing between 100 to 200 ng of DNA were then treated with Exonuclease I (0.5 U/μl) and shrimp alkaline phosphatase (0.1 U/μl) for 15 minutes at 37°C and 15 minutes at 80°C on a 96-well PCR plate. This mixture was used as a template for a cycle sequencing reaction using the DTCS labeling kit from Beckman Coulter Inc. (Fullerton, CA). The primer used for sequencing (PT2F3) is upstream of the inserted cDNA and downstream of the primer PT2F1. Sequencing reaction was performed on a Perkin Elmer 9700 thermacycler. Conditions were 75°C for 2 min, 94°C for 4 min, and 30 cycles of 96°C for 20 sec, 50°C for 20 sec and 60°C

for 4 min. After cycle sequencing the samples, a cleaning step was done using the multi-screen 96 well plate cleaning system from Millipore (Bedford, MA). The 96 well multi-screening plate was prepared by adding a fixed amount (according to the manufacturer's specification) of Sephadex-50 (Amersham Pharmacia Biotech, Piscataway, NJ) and 300 μl of deionized water. After 1 hour of incubation at room temperature, the water was removed from the multi screen plate by centrifugation at 750 g for 5 minutes. After the Sephadex in the multi-screen plate was partially dried, the whole cycle sequencing reaction was added to the center of each well, centrifuged at 750 g for 5 minutes and the clean sample was collected on a sequencing microtiter plate (Beckman Coulter, Fullerton, CA). The plate was then dried on Speed-Vac SC 110 model with a microtiter plate holder (Savant Instruments Inc, Holbrook, NY). The dried samples were immediately resuspended with 25μl of deionized ultrapure formamide (J.T. Baker, Phillipsburg, NJ), and one drop of mineral oil was added to the top of each sample. Samples were sequenced immediately on a CEQ 2000 DNA sequencing instrument (Beckman Coulter Inc., Fullerton, CA) or stored at – 30°C. The entire cDNA of selected genes was fully sequenced using custom primers using a CEQ 2000 DNA sequencing instrument (Beckman Coulter Inc., Fullerton, CA) as described above. The *P. papatasi* salivary gland cDNA library was plated to approximately 200 plaques/plate (150 mm Petri dish), and sequenced as previously described (30).

DNA Vaccine Construction and Description of the VR1020 Vector. The gene coding for SP15 (from N-terminus to stop codon) was amplified from *P. papatasi* SP15-specific cDNA by PCR using High-Fidelity platinum *Taq* polymerase (GIBCO BRL) and specific primers carrying *BamHI* restriction sites (Forward SP15BHF 5'-TGCGGATCCGAAAATCCATCAAAGAAG-3' (SEQ ID NO:883); Reverse SP15BHR 5'-ATTGGATCCTTATATATTGATTGTTTT-3') (SEQ ID NO:884)). The PCR product was immediately cloned into the TOPO TA cloning vector PCRII (Invitrogen) following manufacturer's specifications. The ligation mixture was used to transform TOP10 cells (Invitrogen) and the cells were incubated overnight at 37°C. Eight colonies were picked and mixed with 10 ml of sterile water. Five ml of each sample were transferred to Luria broth with ampicillin (100 mg/ml) and grown at 37°C. The other 5 ml were used as a template for a PCR reaction using two vector-specific

primers from the PCRII vector to confirm the presence of the insert and for sequencing analysis. After visualization of the PCR product on a 1.1% agarose gel, the eight PCR products were completely sequenced as described above using a CEQ2000 DNA sequencing instrument (Beckman Coulter). A sample that contained the sequence from the N-terminus to stop codon of the SP15 gene including the incorporated *BamHI* sites was chosen. Cells containing the plasmid carrying the SP15 gene were grown overnight at 37°C on Luria broth with ampicillin (100 mg/ml), and plasmid isolation was performed using the Wizard Miniprep kit (Promega). The plasmid containing the SP15 gene with incorporated *BamHI* sites was digested with *BamHI* and then ligated with the *BamHI* predigested VR1020 DNA plasmid vector (VICAL). The VR1020 plasmid contains a kanamycin resistance gene, the human cytomegalovirus promoter, and the tissue plasminogen activator signal peptide upstream of the *BamHI* cloning site. The ligation reaction between the *BamHI* digested SP15 gene and the similarly digested VR1020 DNA vector was done overnight at 16°C to transform TOP10 cells (Invitrogen). Cells were incubated on a Luria broth kanamycin (30 mg/ml) plate overnight at 37°C. Thirty-two colonies were picked and mixed with 10 ml of sterile water. Five ml of each sample were transferred to Luria broth with kanamycin (100 mg/ml) and grown at 37°C. The other 5 ml were used as a template for a PCR reaction using two vector-specific primers from the VR1020 vector to confirm the presence of the insert and for sequencing analysis. After visualization of the PCR product on a 1.1% agarose gel, four PCR products were completely sequenced as described above using a CEQ2000 DNA sequencing instrument (Beckman Coulter). For the vaccine construct, a sample that contained the sequence from the N-terminus to the stop codon in the right orientation and in the correct open-reading-frame following the tissue plasminogen activator signal peptide was chosen. Cells containing the SP15 gene on VR1020 were grown overnight at 37°C on Luria broth with kanamycin (100 mg/ml), and plasmid isolation was performed using the Wizard Miniprep kit. After plasmid isolation, the sample and control plasmids (VR1020 alone) were washed three times with ultrapure water using an Amicon-100 (Millipore). The concentrations of the samples were measured by UV absorbance, and they were stored at -70°C before immunization experiments.

DNA and Predicted Protein Sequence Analysis. DNA data derived from the mass sequencing project were analyzed by an in-house program written in VisualBASIC (Microsoft). This program removed vector and primer sequences from the raw sequence. Stripped sequences were compared to the NCBI non-redundant protein database using the program BlastX using the BLOSUM-62 matrix (21). DNA sequences were clustered by blasting the database against itself with a preselected threshold cutoff, usually $1e^{-10}$ (BlastN program) (21). Sequences from the same cluster were aligned using ClustalX (22). To find the cDNA sequences corresponding to the amino acid sequence obtained by Edman degradation of the proteins transferred to PVDF membranes from PAGE gels, a search program was written that checked these amino acid sequences against the three possible protein translations of each cDNA sequence obtained in the mass sequencing project. This was written using the same approach used in the BLOCKS (23) or Prosite (24) programs. Protein translations of the full-length clones were further processed to identify the predicted signal peptides using the Signal P program (25), available at http://genome.cbs.dtu.dk/services/SignalP/. Predicted signal peptide cleaved sites were compared to the N-terminus sequence obtained from Edman degradation of *P. papatasi* salivary proteins. Estimation of isoelectric point and mol wt of translated protein was performed using the DNA STAR program (DNASTAR). Full-length translated protein sequence information was compared with the non-redundant protein database of NCBI using the BLAST-P program (21) and searched for motifs by submitting each sequence to http://www.motif.genome.ad.jp/.

To characterize the primary structure of the main proteins of *P. papatasi* SGH, SDS-PAGE gels were transferred to PVDF membranes, and the amino terminal sequence of each cut band by Edman degradation were estimated. Of 12 bands, with the exception of one that is probably blocked by a pyroglutamyl residue (amino terminal sequence QXXX), 11 bands yielded information as shown in Fig. 2. The amino terminal sequences were used to screen a 3-frame translation of a cDNA database originating from ~600 DNA sequences obtained from randomly picked clones from a unidirectionally cloned salivary gland cDNA library. Matching clones were

subsequently fully sequenced using custom primers. The proteins in Fig. 2 are named according to their mobility in non-reducing SDS-PAGE. A brief description of the clones is enclosed in Table I, with their NCBI accession numbers, best match to the NCBI NR protein database, predicted mol wt, and motifs found. All proteins had a predicted signal secretory sequence (25), and their site of cleavage was confirmed by Edman degradation. All proteins were basic, with a pI ranging from 8.28 to 9.43. Note that the same amino terminal sequence for two SDS-PAGE bands was obtained, located at 32 kD and 64 kD; the heavier is presumed to be a homodimer. Additionally, the same amino terminal sequence was obtained from the SDS-PAGE bands located at 42 kD and 46 kD, the difference possibly arising from post-translation modification.

Parasite Preparation and Intradermal Inoculation. *L. major* clone V1 (MHOM/IL/80/Friedlin) was cultured in 199 medium with 20% heat-inactivated fetal bovine serum (HyClone), 100 U/ml penicillin, 100 mg/ml streptomycin, 2 mM L-glutamine, 40 mM Hepes, 0.1 mM adenine (in 50 mM Hepes), 5 mg/ml hemin (in 50% triethanolamine), and 1 mg/ml 6-biotin (M199/S). Infective-stage metacyclic promastigotes of *L.major* were isolated from stationary cultures (4-5 d old) by negative selection using peanut agglutinin (Vector Labs). Five hundred metacyclic promastigotes with or without 0.5 pair of SGH were inoculated intradermally into the left ear dermis using a 27-gauge needle in a volume of approximately 5 ml. The evolution of the lesion was monitored by measuring the diameter of the induration of the ear lesion with a direct-reading Vernier caliper (Thomas Scientific).

Estimation of Parasite Load. Parasite titrations were performed as previously described (16, 25).

Vaccination. SGH (30 salivary gland pairs) was separated by SDS-PAGE and stained with Coomassie blue as described above. The gel was divided into three groups (bands) containing proteins in the range of 200 to 50 kD (Fraction A, approximately 11 mg), from 49 to 20 kD (Fraction B, approximately 9 mg), and below 20 kD (Fraction C, approximately 10 mg). A control piece of acrylamide from the gel was used (Fraction E). The gel was cut and the bands transferred to a 1.5-ml tube and washed three times with sterile PBS (pH 6.8) plus 150 mM NaCl. The bands were then triturated using a plastic pestle until the preparation could be resuspended in 500 ml of sterile phosphate-

saline buffer using a 27-gauge needle to give a solution of approximately 0.02 mg/ml. Immunization with SDS-PAGE fractions was carried out by the injection of 10 ml of either Fraction A, B, C, or E into the right ear of each mouse, followed by a boost 2 wk later. For genetic immunization, mice were inoculated in the right ear with 5–10 mg of the plasmid encoding for SP15 or control DNA (empty vector) suspended in 5 ml of PBS. Each group was boosted 2 wk later using the same regimen. Mice were bled and the presence of antibodies assessed for each individual mouse by Western blot as described above.

Analysis of the Inflammatory Response in the Ear Dermis. The left ears of mice vaccinated with the plasmid vector (in the right ear) or not vaccinated were exposed to the bite of sand flies as previously described (19). Twenty-four h following the sand fly exposure, seven mice per group were sacrificed and the left ear collected. Each ear was processed individually. The cells in the inflammatory ear dermis were recovered as previously described (17).

Immunolabeling for Flow Cytometry Analysis. The dermal cells were incubated with 10% normal mouse serum in PBS containing 0.1% BSA, 0.01% NaN_3 before being incubated with anti-Fc receptor antibody (2.4G2, PharMingen). The double staining was done by using directly conjugated antibodies incubated simultaneously. The dermal inflammatory cells were identified by characteristic size, forward scatter (FSC) and granulosity, side scatter (SSC) combined with two-color analysis, as previously described (27).

Statistical Analysis. Statistical tests were performed with SigmaStat (Jandel Software, San Rafael, CA). Because most comparisons derived from data with non-homogeneous variances, Kruskal-Wallis ANOVA on ranks was performed and multiple comparisons were done by the Dunn method. Dual comparisons were made with the Mann-Whitney rank sum test. All data from parasite numbers were log-transformed prior to conducting statistical tests.

Immune Responses. To investigate which fractions from saliva produce the most effective protection when mice are subsequently challenged with parasites and SGH, SGH was separated by SDS-PAGE, and the gel was divided into three groups containing proteins in the range of 200 kD to 40 kD, from 39 kD to 20 kD, and below

20 kD. A control group receiving gel without proteins was also run, together with non-injected controls. After intradermal immunization in the ear, mice were challenged in the contralateral ear with parasites and SGH. Naïve mice inoculated with SGH plus parasites had significantly increased lesion size at 5.5 weeks post inoculation, when compared with naïve mice inoculated with parasites only. Mice vaccinated with the lower mol wt fractions (Fraction C), and challenged with both parasites and SGH had the best protection in this assay (Fig. 3 A) ($P < 0.05$). Parasite load at 4.5 wk post inoculation were also lower in fraction C-vaccinated mice ($P < 0.05$) (Fig. 3 B). A strong antibody response was obtained when mice were inoculated with the smaller protein size region of the gel (Fig. 4). Within this group, a stronger reaction was mounted against an antigen identical to or co-migrating with a protein of 15 kD, here named SP15 protein (PpSP15), as identified in Fig. 2 and Table I.

To test the role of the SP15 gel region in conferring resistance to leishmaniasis in mice, mice were vaccinated with the SP15 band obtained from SDS-PAGE or with an acrylamide control injection and challenged with *L. major* in the presence and absence of SGH. Results indicated that vaccination with the SP15 band greatly affected disease manifestation as measured by lesion size, which were significantly smaller ($P < 0.05$) than those of mice vaccinated but not receiving SGH at the time of *Leishmania* inoculation or of acrylamide-vaccinated mice receiving parasites plus SGH (Fig. 5 A). The parasite load at 9-wk post inoculation was smaller on the SP15-vaccinated mice challenged with parasites and SGH, but only borderline so ($P = 0.056$) when compared with the acrylamide-vaccinated controls (Fig. 5 B). This may be explained by the presence of acrylamide in the preparation. Acrylamide was used as an adjuvant, and this may be the reason for these results (acrylamide confers some level of protection). When these experiments were repeated using DNA vaccination, the lesion size and the parasite number were significantly lower in the SP15 DNA-vaccinated mice as compared to control DNA-vaccinated mice.

To further confirm whether immunity against SP15 could protect mice when parasites are co-injected with SGH, a DNA vaccine was constructed using the *P. papatasi* salivary SP15 gene. The SP15 cDNA is 522 bp in length and codes for a protein of 142 amino acids including the signal peptide. The mature protein, as

predicted by the Signal P program and confirmed by Edman degradation, has 14,494.81 dalton. Accordingly, the SP15 gene coding for the mature protein was inserted downstream and in frame with the signal peptide of tissue plasminogen activator present in the VR1020 vector. This construct is named SP15-Pl. Control plasmids, consisting of VR1020 plasmid alone, were used to immunize control mice. This control plasmid is named CTL-Pl. Mice immunized with SP15-Pl, but not those immunized with the CTL-Pl, produced antibodies recognizing a single band in Western blots of SGH, at the position of SP15 in the gel (Fig. 6 A). Additionally, to determine whether the SP15 vaccination would induce a DTH response in mice following the bite of uninfected sand flies, naïve, SP15-Pl-immunized, and plasmid control-immunized mice were exposed to sand fly bites. Results indicate that mice vaccinated with SP15-Pl developed an intense DTH response 24 h following exposure to sand flies (Fig. 6 B). This reaction is characterized by swelling and a massive cell infiltrate consisting primarily of neutrophils, eosinophils, and macrophages (Fig. 6 B). This experiment indicates that the plasmid vaccine is producing both serum and cellular immunity reactions.

The effects of immunization with SP15-Pl on the development of *L. major* infection co-injected or not with SGH were investigated. The intradermal co-inoculation of 500 *L. major* with 0.5 pair of SGH had a dramatic effect on the pathology in naïve mice or mice vaccinated with CTL-Pl (Fig. 7). The lesions developed more rapidly and presented an ulcerative pathology compared with controls inoculated without saliva. In contrast, mice previously vaccinated with SP15-Pl and challenged with parasite plus SGH developed a minor and non-ulcerative pathology. The lesions were significantly smaller not only compared with controls (naïve or CTL-Pl) inoculated with saliva but also compared with mice inoculated with the parasite alone. The induration in the SP15-Pl-vaccinated mice resolved as early as 6 wk post challenge, while in the CTL-Pl-immunized mice inoculated with saliva, the large ulcerative, necrotic lesions are maintained. Similarly, the intradermal co-inoculation of 500 *L. major* with 0.5 pair of SGH enhanced the parasite load in naïve and CTL-Pl vaccinated C57BL/6 mice compared with their respective controls, inoculated with the parasite alone (Fig. 7).

To evaluate the persistence of the immunity induced by the DNA vaccine, the animals were challenged or not with SGH 12 wk, rather than 2 wk, after the last boosting. The protection was comparable to that achieved when the challenge was performed 2 wk post vaccination, with a significant reduction of both lesion size and parasite number (Fig. 8).

Having shown that immunization with SP15-Pl induces both antibody and DTH (Fig. 6) responses, the contribution of antibodies versus cellular immunity in the anti-*Leishmania* protective effect was evaluated. $B^{-/-}$ mice were immunized with SP15-Pl and CTL-Pl with the expectation that this model animal would provide only a cellular response to SP15. Indeed, similarly to the wild-type (WT) controls only $B^{-/-}$ mice vaccinated with SP15-Pl developed an intense DTH response when challenged with 500 *L. major* promastigotes in the presence of 0.5 pairs of SGH (Fig. 9 B). As expected, no antibodies from the $B^{-/-}$ mice were detected by Western blots, (Fig. 9 A), although antibodies were produced by the control WT mice (B10 mice). When challenged with parasites and SGH, vaccinated $B^{-/-}$ mice had significantly lower pathology at 5.5 wk post inoculation than did mice vaccinated with the control plasmid ($P = 0.008$) (Fig. 10 A). When compared with the CTL-Pl-vaccinated mice ($P < 0.05$), parasite loads at 5.5 wk post inoculation were significantly lower in mice vaccinated with SP15-Pl and inoculated with parasites plus SGH, irrespective of whether the mice were B cell deficient (Fig. 10 B). Comparisons of the parasite loads between $B^{-/-}$ and WT mice indicated that $B^{-/-}$ mice had smaller parasite loads than the WT controls ($P = 0.006$).

Although the present process has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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What is claimed is:

1. An isolated nucleic acid, encoding a salivary polypeptide of *Phlebotomus papatasi*.

2. The nucleic acid of claim 1, encoding the salivary polypeptide having the N-terminal sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9 .

3. The nucleic acid of claim 1, encoding the salivary polypeptide of *Phlebotomus papatasi* having the amino acid sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:18.

4. The nucleic acid of claim 3, wherein the nucleic acid comprises the nucleotide sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26 and SEQ ID NO:27.

5. An isolated fragment of the nucleic acid of claim 1, wherein the fragment encodes a polypeptide fragment specific for the polypeptide having the amino acid sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:18.

6. An isolated salivary polypeptide of *Phlebotomus papatasi*.

7. The isolated polypeptide of claim 6, wherein the polypeptide is the salivary polypeptide having the N-terminal sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9 .

8. The isolated polypeptide of claim 7 having the amino acid sequence selected from the group consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:18.

9. An isolated antigenic or immunogenic fragment of the polypeptide of claim 6.

10. The isolated antigenic or immunogenic fragment of claim 9, having the amino acid sequence of SEQ ID NOS:28-880.

11. A nucleic acid that hybridizes under stringent conditions to the nucleic acids of SEQ ID NOS:19-27.

12. The nucleic acid of claim 11, wherein the nucleic acid encodes an antigenic or immunogenic polypeptide.

13. The nucleic acid of claim 1 in a vector.

14. The nucleic acid of claim 2 in a vector.

15. The nucleic acid of claim 3 in a vector.

16. The nucleic acid of claim 4 in a vector.

17. The nucleic acid of claim 5 in a vector.

18. A vector comprising at least one nucleic acid or fragment thereof, selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26 and SEQ ID NO:27.

19. A composition comprising the vector of claim 13 and a pharmaceutically acceptable carrier.

20. A composition comprising the vector of claim 14 and a pharmaceutically acceptable carrier.

21. A composition comprising the vector of claim 15 and a pharmaceutically acceptable carrier.

22. A composition comprising the vector of claim 16 and a pharmaceutically acceptable carrier.

23. A composition comprising the vector of claim 17 and a pharmaceutically acceptable carrier.

24. A composition comprising the vector of claim 18 and a pharmaceutically acceptable carrier.

25. A composition comprising the polypeptide of claim 6 or fragment thereof and a pharmaceutically acceptable carrier.

26. A composition comprising the polypeptide of claim 7 or fragment thereof and a pharmaceutically acceptable carrier.

27. A composition comprising the polypeptide of claim 8 or fragment thereof and a pharmaceutically acceptable carrier.

28. A composition comprising the polypeptide of claim 9 or fragment thereof and a pharmaceutically acceptable carrier.

29. A composition comprising the fragment of claim 10 and a pharmaceutically acceptable carrier.

30. A composition comprising at least one polypeptide or fragment thereof, selected from the group having the amino acid sequence of SEQ ID NO:10, SEQ ID NO:11,

SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:18 and a pharmaceutically acceptable carrier.

31. A method of producing an immune response in a subject, comprising administering to the subject an effective amount of the composition of claim 19, 20, 21, 22, 23 or 24.

32. A method of producing an immune response in a subject, comprising administering to the subject an effective amount of the composition of claim 25, 26, 27, 28 or 29.

33. A method of preventing Leishmaniasis in a subject, comprising administering to the subject an effective amount of the composition of claim 19, 20, 21, 22, 23 or 24.

34. A method of preventing Leishmaniasis in a subject, comprising administering to the subject an effective amount of the composition of claim 25, 26, 27, 28 or 29.

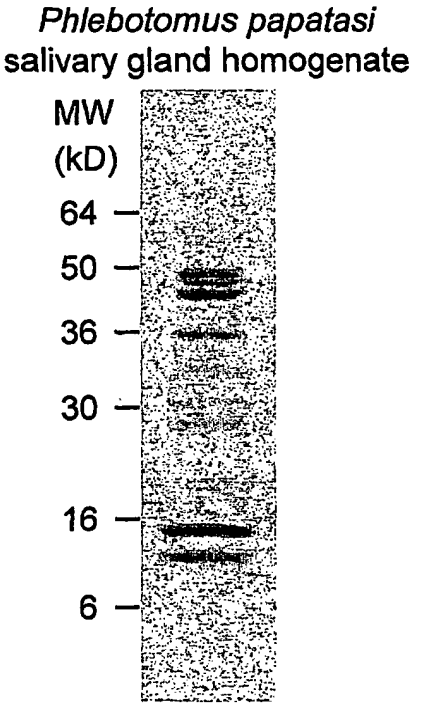

Phlebotomus papatasi
salivary gland homogenate
MW
(kD)
64
50
36
30
16
6

FIG.1A

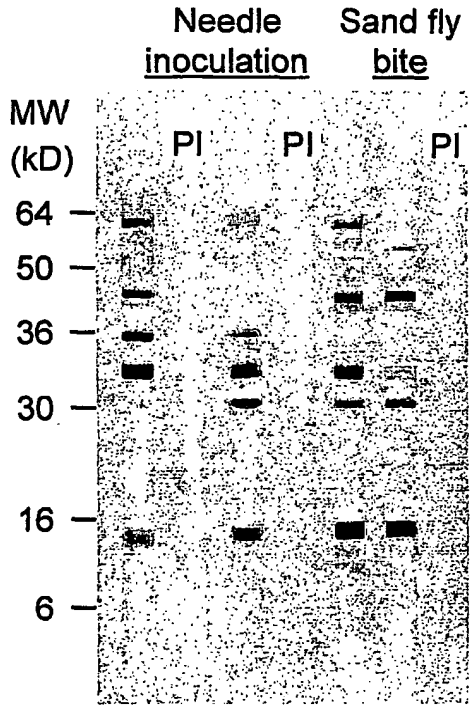
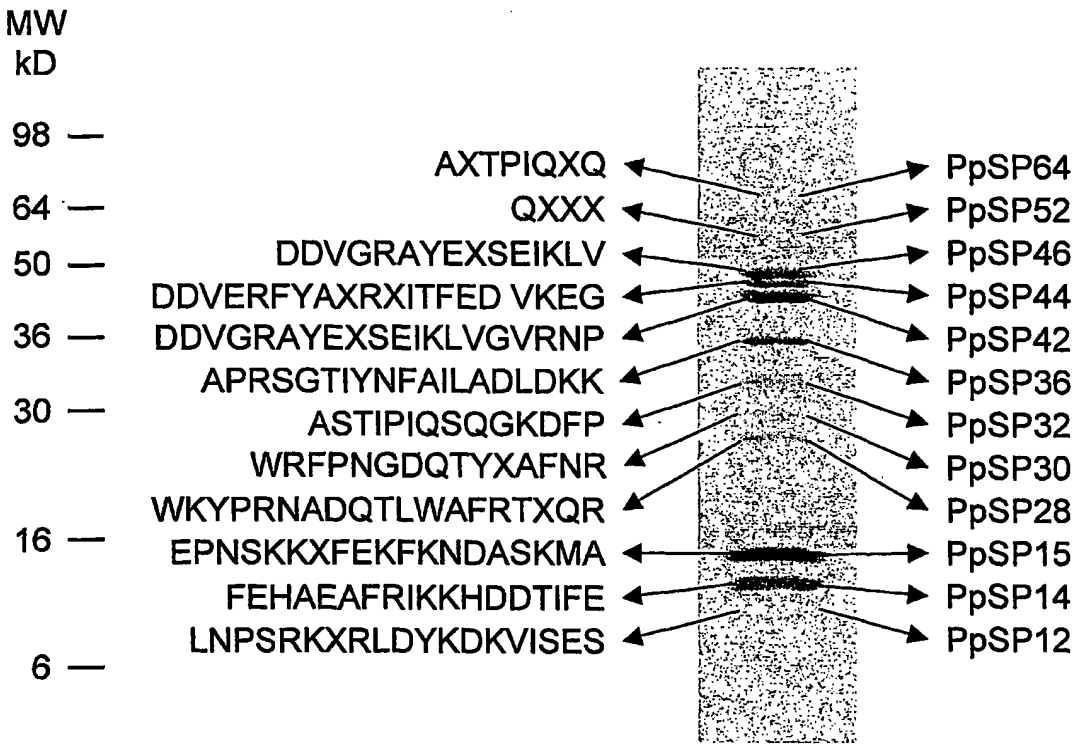

Needle
inoculation
Sand fly
bite
MW
(kD)
PI
PI
PI
64
50
36
30
16
6

FIG.1B


MW
kD
98
64
50
36
30
16
6
AXTPIQXQ
QXXX
DDVGRAYEXSEIKLV
DDVERFYAXRXITFED VKEG
DDVGRAYEXSEIKLVGVRNP
APRSGTIYNFAILADLDKK
ASTIPIQSQGKDFP
WRFPNGDQTYXAFNR
WKYPRNADQTLWAFRTXQR
EPNSKKXFEKFKNDASKMA
FEHAEAFRIKKHDDTIFE
LNPSRKXRLDYKDKVISES
PpSP64
PpSP52
PpSP46
PpSP44
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PpSP36
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PpSP30
PpSP28
PpSP15
PpSP14
PpSP12

FIG.2

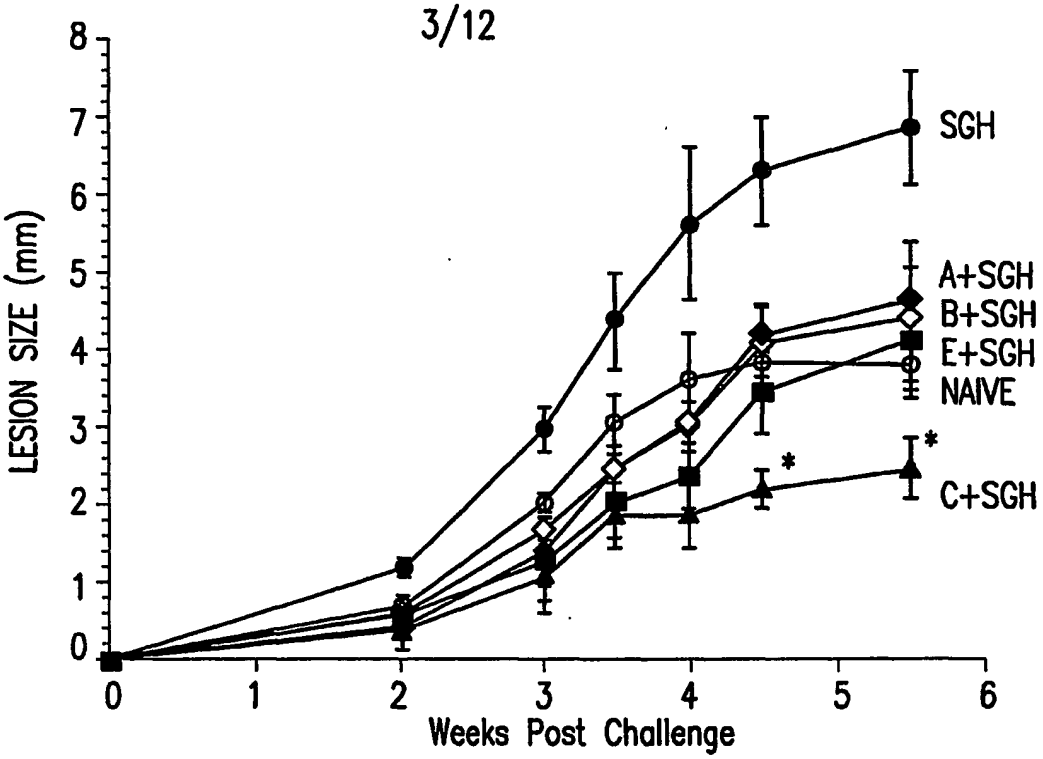
LESION SIZE (mm)
Weeks Post Challenge
SGH
A+SGH
B+SGH
E+SGH
NAIVE
C+SGH
*
*

FIG.3A

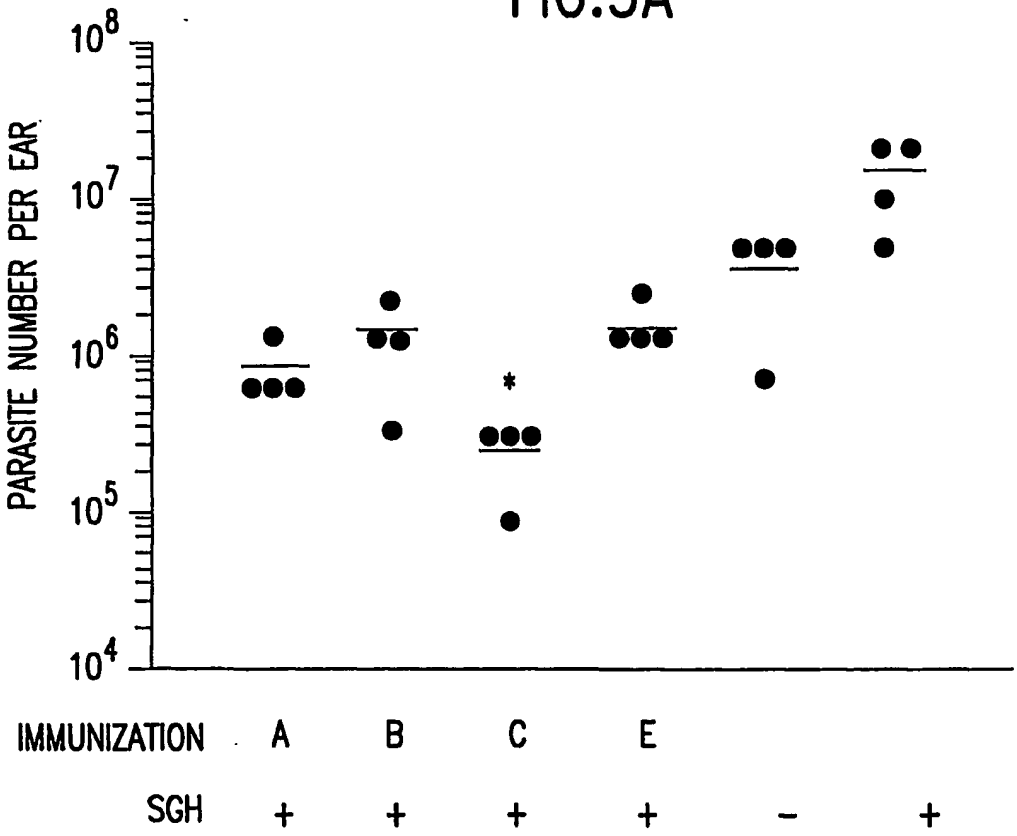
PARASITE NUMBER PER EAR
IMMUNIZATION
A
B
C
E
SGH
+
+
+
+
-
+
*

FIG.3B

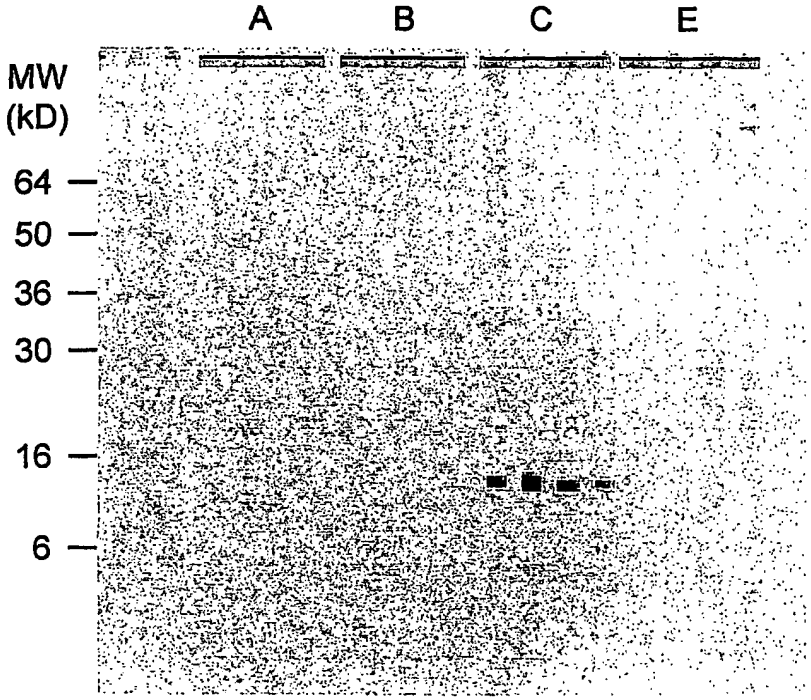

FIG.4

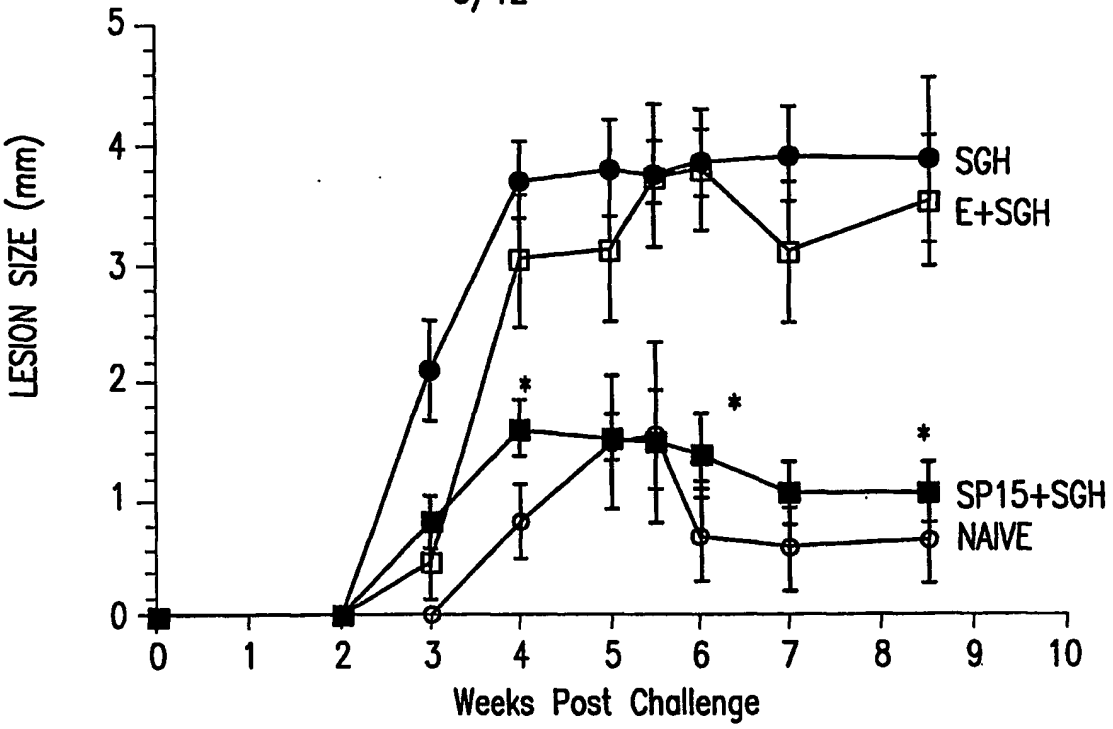
LESION SIZE (mm)
0
1
2
3
4
5
0
1
2
3
4
5
6
7
8
9
10
Weeks Post Challenge
SGH
E+SGH
SP15+SGH
NAIVE
*
*
*

FIG.5A

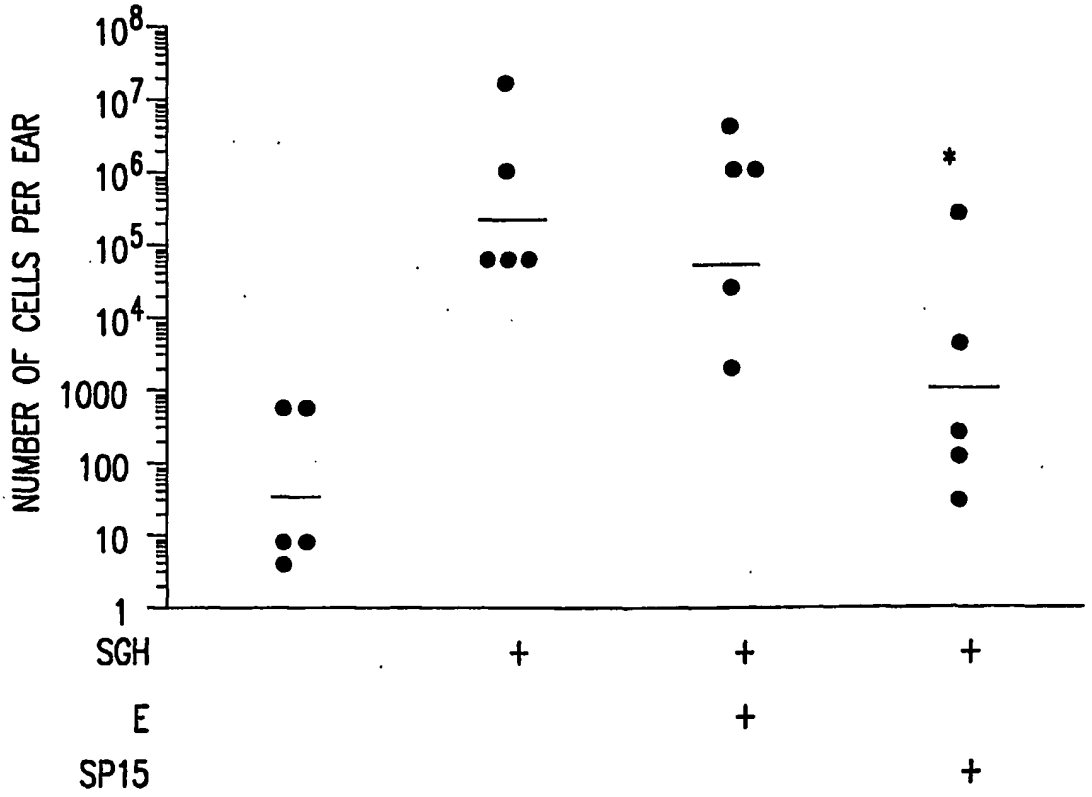
NUMBER OF CELLS PER EAR
10^8
10^7
10^6
10^5
10^4
1000
100
10
1
SGH + + +
E + +
SP15 +
*

FIG.5B

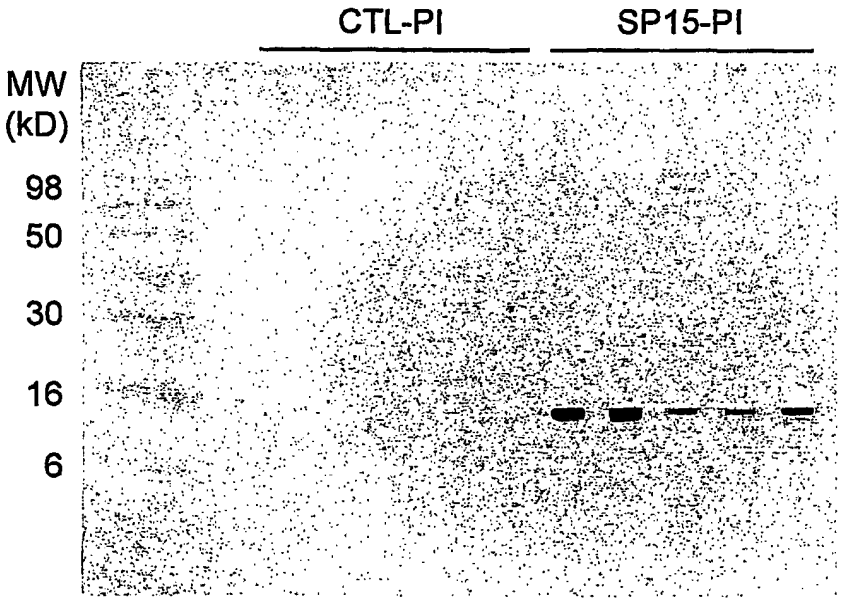

FIG.6A

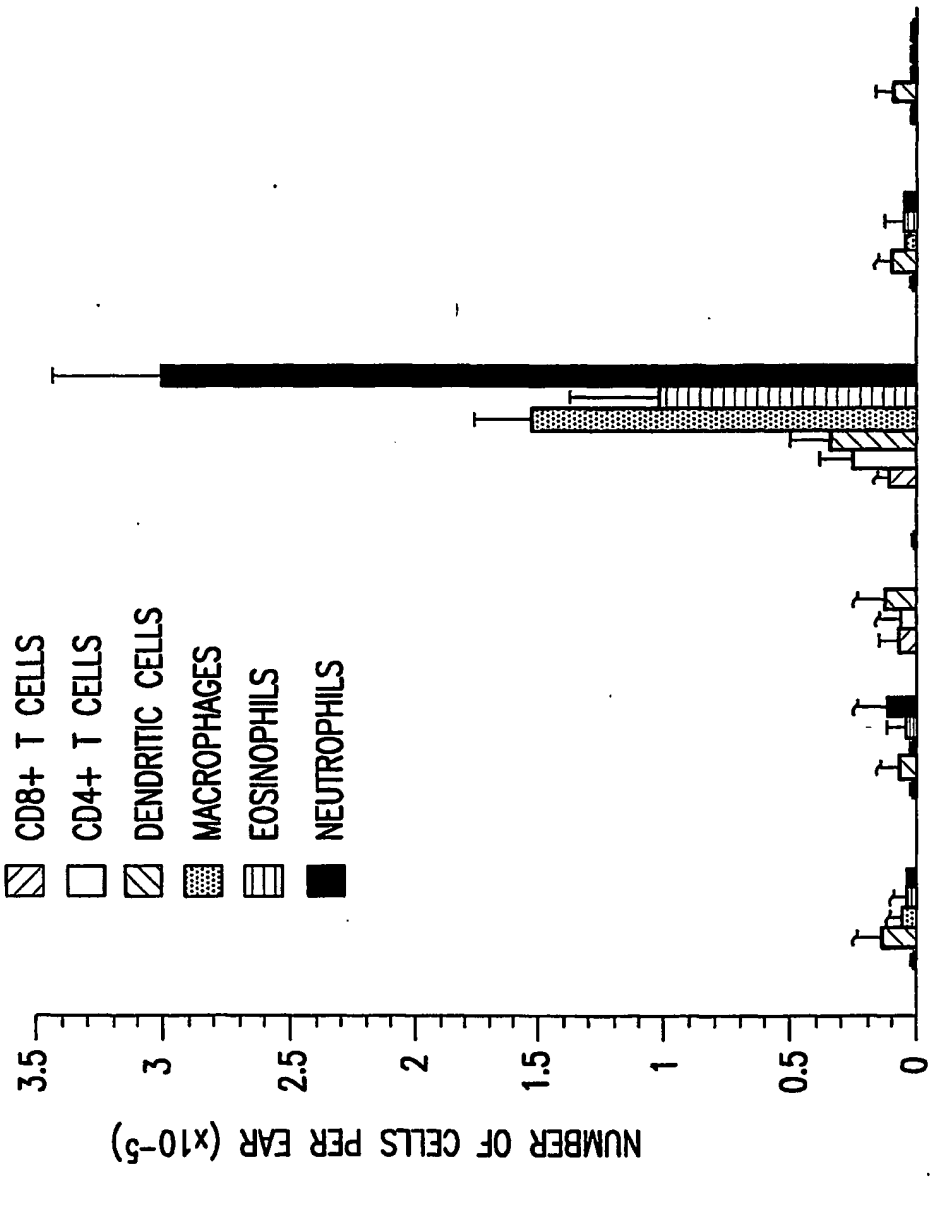
NUMBER OF CELLS PER EAR (x10-5)
3.5
3
2.5
2
1.5
1
0.5
0
CD8+ T CELLS
CD4+ T CELLS
DENDRITIC CELLS
MACROPHAGES
EOSINOPHILS
NEUTROPHILS
IMMUNIZATION - -SP15-P1 SP15-P1 CTL-P1 CTL-P1
CHALLENGE BY BITE - + - + - +

FIG.6B

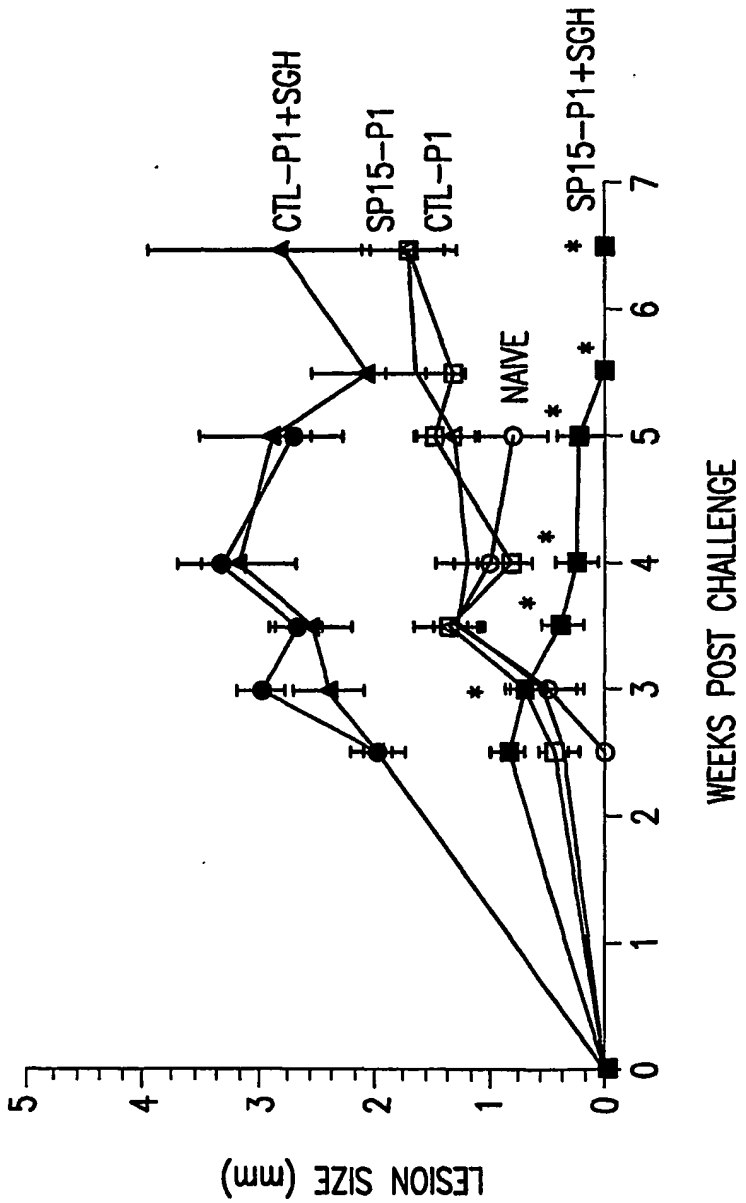
CTL-P1+SGH
SP15-P1
CTL-P1
NAIVE
SP15-P1+SGH
LESION SIZE (mm)
WEEKS POST CHALLENGE
0
1
2
3
4
5
6
7

FIG.7

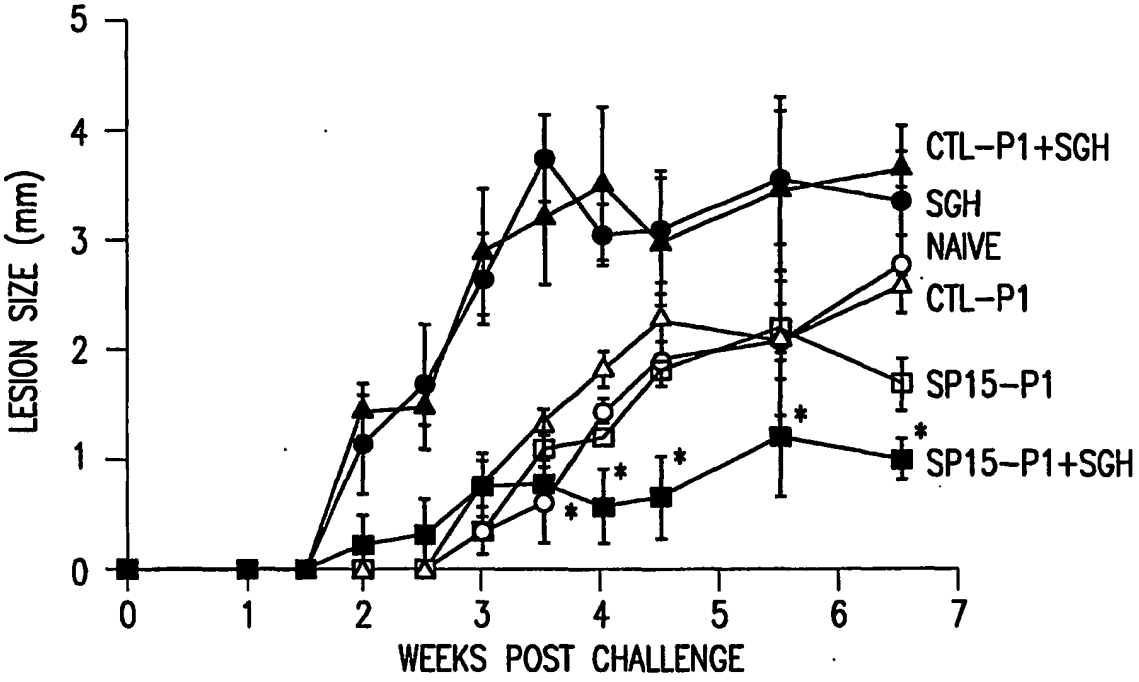
LESION SIZE (mm)
WEEKS POST CHALLENGE
CTL-P1+SGH
SGH
NAIVE
CTL-P1
SP15-P1
SP15-P1+SGH

FIG.8A

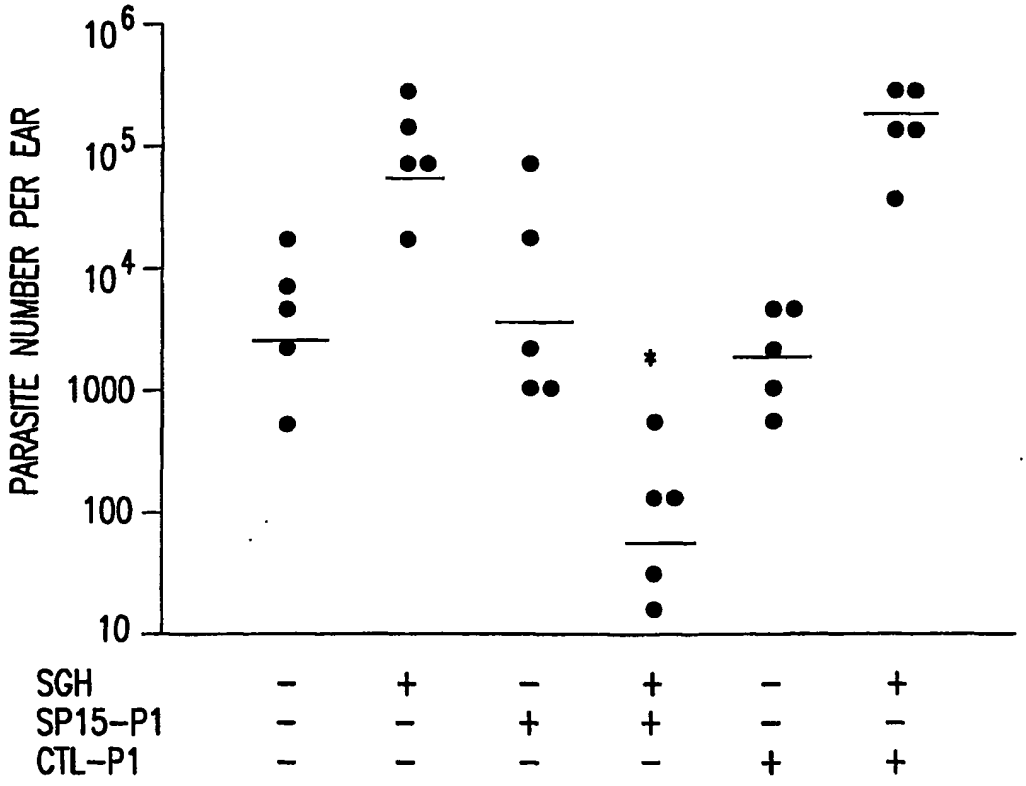
PARASITE NUMBER PER EAR
SGH
SP15-P1
CTL-P1

FIG.8B

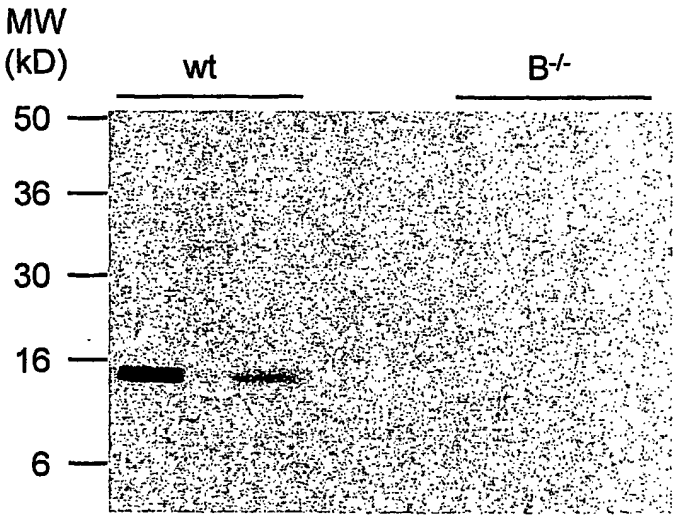

FIG.9A

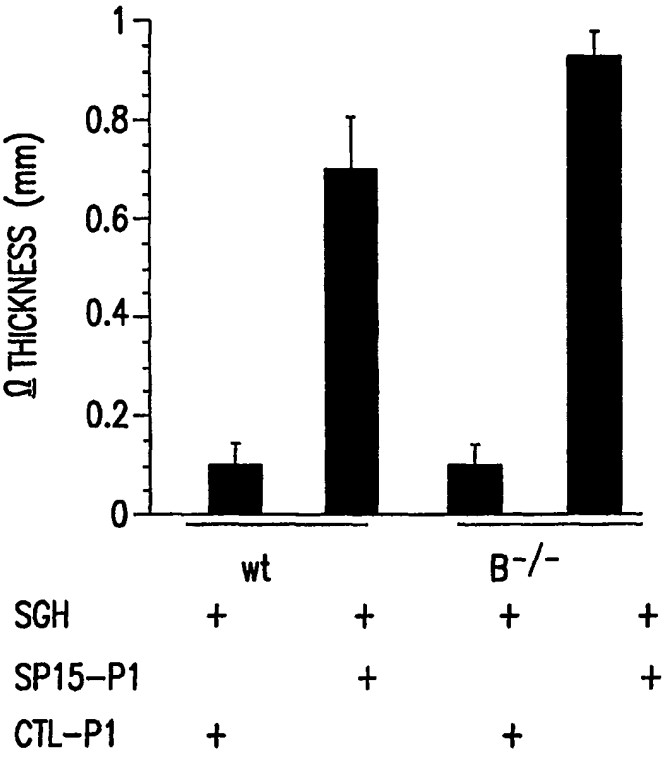

FIG.9B

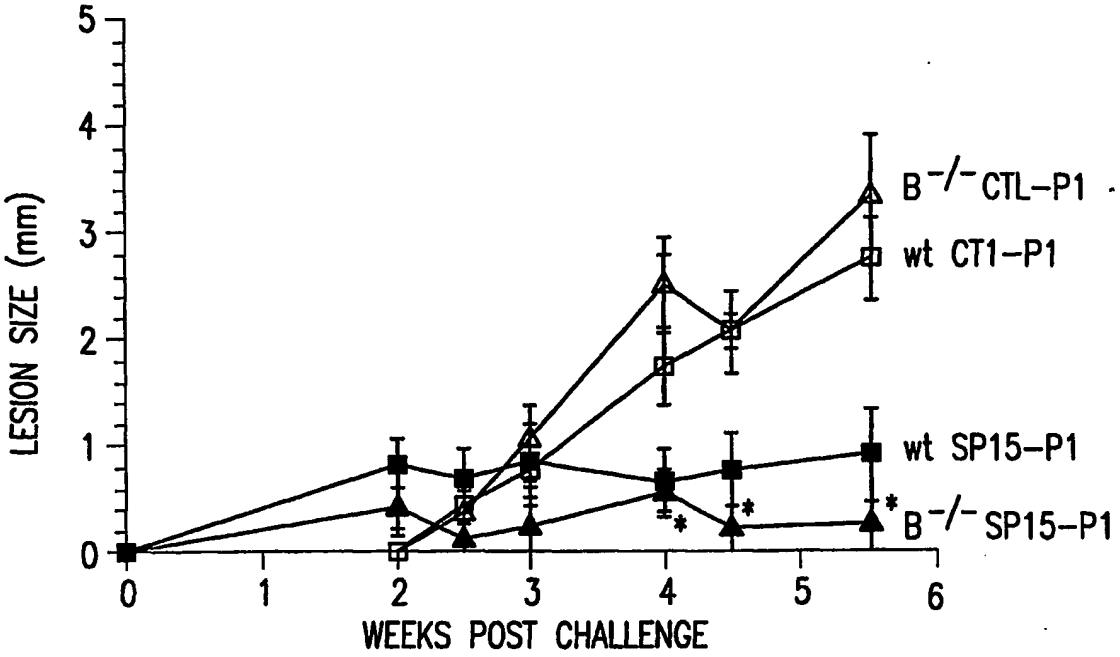

FIG.10A

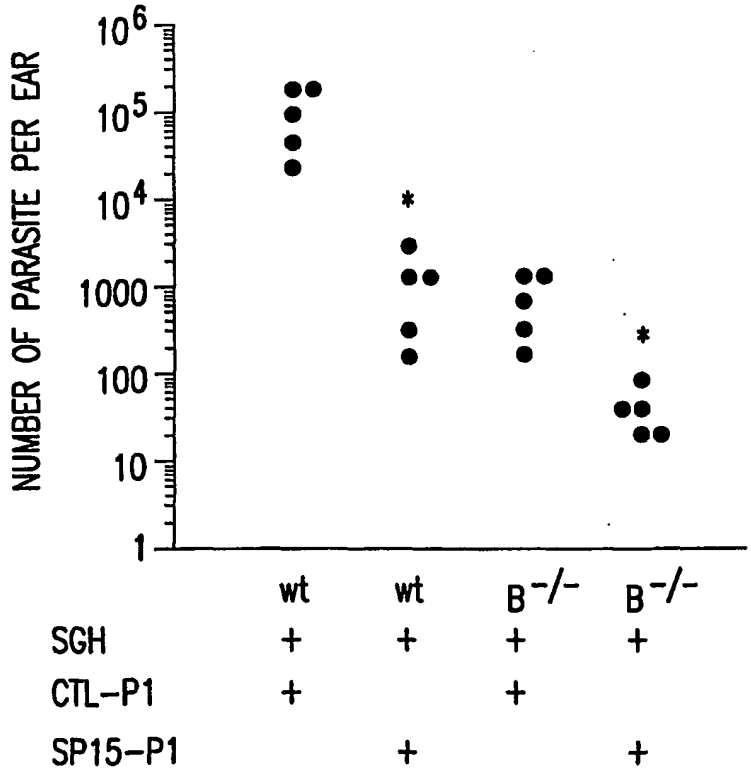

FIG.10B